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RESEARCH INSTITUTE, NEW DELHI.

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OF SCIENCES

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THE ORGANIZATION OF THE NEW YORK ACADEMY OF SCIENCES

THE ORIGINAL CHARTER

AN ACT TO INCORPORATE THE LYCEUM OF NATURAL HISTORY IN THE CITY OF NEW YORK

Passed April 20, 1818

WHEREAS, The members of the Lyceum of Natural History have petitioned for an act of incorporation, and the Legislature, impressed with the importance of the study of Natural History, as connected with the wants, the comforts and the happiness of mankind, and conceiving it their duty to encourage all laudable attempts to promote the progress of science in this State—therefore,

1. *Be it enacted by the People of the State of New York represented in Senate and Assembly*, That Samuel L. Mitchill, Casper W. Eddy, Frederick C. Schaeffer, Nathaniel Paulding, William Cooper, Benjamin P. Kissam, John Torrey, William Cumberland, D'Jurco V. Knevels, James Clements and James Pierce, and such other persons as now are, and may from time to time become members, shall be, and hereby are constituted a body corporate and politic, by the name of LYCEUM OF NATURAL HISTORY IN THE CITY OF NEW YORK, and that by that name they shall have perpetual succession, and shall be persons capable of suing and being sued, pleaded and being impleaded, answering and being answered unto, defending and being defended, in all courts and places whatsoever; and may have a common seal, with power to alter the same from time to time; and shall be capable of purchasing, taking, holding, and enjoying to them and their successors, any real estate in fee simple or otherwise, and any goods, chattels, and personal estate, and of selling, leasing, or otherwise disposing of said real or personal estate, or any part thereof, at their will and pleasure: *Provided always*, that the clear annual value or income of such real or personal estate shall not exceed the sum of five thousand dollars: *Provided*, however, that the funds of the said Corporation shall be used and appropriated to the promotion of the objects stated in the preamble to this act, and those only.

2. *And be it further enacted*, That the said Society shall from time to time, forever hereafter, have power to make, constitute, ordain, and establish such by-laws and regulations as they shall judge proper, for the election of their officers; for prescribing their respective functions, and the mode of discharging the same; for the admission of new mem-

bers; for the government of the officers and members thereof; for collecting annual contributions from the members towards the funds thereof; for regulating the times and places of meeting of the said Society; for suspending or expelling such members as shall neglect or refuse to comply with the by-laws or regulations, and for the managing or directing the affairs and concerns of the said Society: *Provided* such by-laws and regulations be not repugnant to the Constitution and laws of this State or of the United States.

3. *And be it further enacted*, That the officers of the said Society shall consist of a President and two Vice-Presidents, a Corresponding Secretary, a Recording Secretary, a Treasurer, and five Curators, and such other officers as the Society may judge necessary; who shall be annually chosen, and who shall continue in office for one year, or until others be elected in their stead; that if the annual election shall not be held at any of the days for that purpose appointed, it shall be lawful to make such election at any other day; and that five members of the said Society, assembling at the place and time designated for that purpose by any by-law or regulation of the Society, shall constitute a legal meeting thereof.

4. *And be it further enacted*, That Samuel L. Mitchill shall be the President; Casper W. Eddy the First Vice-President; Frederick C. Schaeffer the Second Vice-President; Nathaniel Paulding, Corresponding Secretary; William Cooper, Recording Secretary; Benjamin P. Kissam, Treasurer, and John Torrey, William Cumberland, D'Jurco V. Knevels, James Clements, and James Pierce, Curators; severally to be the first officers of the said Corporation, who shall hold their respective offices until the twenty-third day of February next, and until others shall be chosen in their places.

5. *And be it further enacted*, That the present Constitution of the said Association shall, after passing of this Act, continue to be the Constitution thereof; and that no alteration shall be made therein, unless by a vote to that effect of three-fourths of the resident members, and upon the request in writing of one-third of such resident members, and submitted at least one month before any vote shall be taken thereupon.

State of New York, Secretary's Office.

I CERTIFY the preceding to be a true copy of an original Act of the Legislature of this State, on file in this Office.

ARCH'D CAMPBELL,
Dep. Sec'y.

ALBANY, April, 29, 1818.

ORDER OF COURT

ORDER OF THE SUPREME COURT OF THE STATE OF NEW YORK TO CHANGE THE NAME OF THE LYCEUM OF NATURAL HISTORY IN THE CITY OF NEW YORK TO THE NEW YORK ACADEMY OF SCIENCES

WHEREAS, in pursuance of the vote and proceedings of this Corporation to change the corporate name thereof from "The Lyceum of Natural History in the City of New York" to "The New York Academy of Sciences," which vote and proceedings appear to record, an application has been made in behalf of said Corporation to the Supreme Court of the State of New York to legalize and authorize such change, according to the statute in such case provided, by Chittenden & Hubbard, acting as the attorneys of the Corporation, and the said Supreme Court, on the 5th day of January, 1876, made the following order upon such application in the premises, viz:

At a special term of the Supreme Court of the State of New York, held at the Chambers thereof, in the County Court House, in the City of New York, the 5th day of January, 1876:

Present—HON. GEO. C. BARRETT, *Justice*.

In the matter of the application of
the Lyceum of Natural History
in the City of New York to au-
thorize it to assume the corporate
name of the New York Academy
of Sciences.

On reading and filing the petition of the Lyceum of Natural History in the City of New York, duly verified by John S. Newberry, the President and chief officer of said Corporation, to authorize it to assume the corporate name of the New York Academy of Sciences, duly setting

forth the grounds of said application, and on reading and filing the affidavit of Geo. W. Quackenbush, showing that notice of such application had been duly published for six weeks in the State paper, to wit, *The Albany Evening Journal*, and the affidavit of David S. Owen, showing that notice of such application has also been duly published in the proper newspaper of the County of New York, in which county said Corporation had its business office, to wit, in *The Daily Register*, by which it appears to my satisfaction that such notice has been so published, and on reading and filing the affidavits of Robert H. Browne and J. S. Newberry, thereunto annexed, by which it appears to my satisfaction that the application is made in pursuance of a resolution of the managers of said Corporation to that end named, and there appearing to me to be no reasonable objection to said Corporation so changing its name as prayed in said petition: Now on motion of Grosvenor S. Hubbard, of Counsel for Petitioner, it is

Ordered, That the Lyceum of Natural History in the City of New York be and is hereby authorized to assume the corporate name of The New York Academy of Sciences.

Indorsed: Filed January 5, 1876,

A copy.

WM. WALSH, Clerk.

Resolution of The Academy accepting the order of the Court, passed February 21, 1876

And whereas, The order hath been published as therein required, and all the proceedings necessary to carry out the same have been had, Therefore:

Resolved, That the foregoing order be and the same is hereby accepted and adopted by this Corporation, and that in conformity therewith the corporate name thereof, from and after the adoption of the vote and resolution herein above referred to, be and the same is hereby declared to be THE NEW YORK ACADEMY OF SCIENCES.

AMENDED CHARTER

MARCH 19, 1902

CHAPTER 181 OF THE LAWS OF 1902

AN ACT to amend chapter one hundred and ninety-seven of the laws of eighteen hundred and eighteen, entitled "An act to incorporate the Lyceum of Natural History in the City of New York," a Corporation now known as The New York Academy of Sciences and to extend the powers of said Corporation.

(Became a law March 19, 1902, with the approval of the Governor. Passed, three-fifths being present.)

The People of the State of New York, represented in Senate and Assembly, do enact as follows:

SECTION I. The Corporation incorporated by chapter one hundred and ninety-seven of the laws of eighteen hundred and eighteen, entitled "An act to incorporate the Lyceum of Natural History in the City of New York," and formerly known by that name, but now known as The New York Academy of Sciences through change of name pursuant to order made by the supreme court at the city and county of New York, on January fifth, eighteen hundred and seventy-six, is hereby authorized and empowered to raise money for, and to erect and maintain, a building in the city of New York for its use, and in which also at its option other scientific societies may be admitted and have their headquarters upon such terms as said Corporation may make with them, portions of which building may be also rented out by said Corporation for any lawful uses for the purposes of obtaining income for the maintenance of such building and for the promotion of the objects of the Corporation; to establish, own, equip, and administer a public library, and a museum having especial reference to scientific subjects; to publish communications, transactions, scientific works, and periodicals; to give scientific instruction by lectures or otherwise; to encourage the advancement of scientific research and discovery, by gifts of money, prizes, or other assistance thereto. The building, or rooms, of said Corporation in the City of New York used exclusively for library or scientific purposes shall be subject to the provisions and be entitled to the benefits of subdivision seven of section four of chapter nine hundred and eight of the laws of eighteen hundred and ninety-six, as amended.

SECTION II. The said Corporation shall from time to time forever hereafter have power to make, constitute, ordain, and establish such by-laws and regulations as it shall judge proper for the election of its officers; for prescribing their respective functions, and the mode of discharging the same; for the admission of new members; for the government of officers and members thereof; for collecting dues and contributions towards the funds thereof; for regulating the times and places of meeting of said Corporation; for suspending or expelling such members as shall neglect or refuse to comply with the by-laws or regulations, and for managing or directing the affairs or concerns of the said Corporation: and may from time to time alter or modify its constitution, by-laws, rules, and regulations.

SECTION III. The officers of the said Corporation shall consist of a president and two or more vice-presidents, a corresponding secretary, a recording secretary, a treasurer, and such other officers as the Corporation may judge necessary; who shall be chosen in the manner and for the terms prescribed by the constitution of the said Corporation.

SECTION IV. The present constitution of the said Corporation shall, after the passage of this act, continue to be the constitution thereof until amended as herein provided. Such constitution as may be adopted by a vote of not less than three-quarters of such resident members and fellows of the said New York Academy of Sciences as shall be present at a meeting thereof, called by the Recording Secretary for that purpose, within forty days after the passage of this act, by written notice duly mailed, postage prepaid, and addressed to each fellow and resident member at least ten days before such meeting, at his last known place of residence, with street and number when known, which meeting shall be held within three months after the passage of this act, shall be thereafter the constitution of the said New York Academy of Sciences, subject to alteration or amendment in the manner provided by such constitution.

SECTION V. The said Corporation shall have power to consolidate, to unite, to co-operate, or to ally itself with any other society or association in the city of New York organized for the promotion of the knowledge or the study of any science, or of research therein, and for this purpose to receive, hold, and administer real and personal property for the uses of such consolidation, union, co-operation, or alliance subject to such terms and regulations as may be agreed upon with such associations or societies.

SECTION VI. This act shall take effect immediately.

STATE OF NEW YORK,

OFFICE OF THE SECRETARY OF STATE.

I have compared the preceding with the original law on file in this office, and do hereby certify that the same is a correct transcript therefrom, and the whole of said original law.

Given under my hand and the seal of office of the Secretary of State, at the city of Albany, this eighth day of April, in the year one thousand nine hundred and two.

JOHN T. McDONOUGH,
Secretary of State.

CONSTITUTION

ADOPTED, APRIL 24, 1902, AND AMENDED AT SUBSEQUENT TIMES

ARTICLE I. The name of this Corporation shall be The New York Academy of Sciences. Its object shall be the advancement and diffusion of scientific knowledge, and the center of its activities shall be in the City of New York.

ARTICLE II. The Academy shall consist of eight classes of members, namely: Sustaining Members, Active Members, Fellows, Associate Members, Student Members, Affiliated Members, Corresponding Members, and Honorary Members. Sustaining and Active Members shall be the members of the Corporation who live in or near the City of New York, or who, having removed to a distance, desire to retain their connection with the Academy. Fellows shall be chosen from the Sustaining and Active Members in virtue of their scientific attainments. Associate Members are members living at a distance from the City of New York, who wish to maintain a membership connection with the Academy without the responsibility and privileges of Active Membership. Student Members are junior workers in science connected with scientific institutions, as regular or research students, or junior faculty members, who wish temporary membership in the Academy. Corresponding and Honorary Members shall be chosen from among persons who have attained distinction in some branch of science. The number of Corresponding Members shall not exceed two hundred, and the number of Honorary Members shall not exceed fifty.

ARTICLE III. None but Fellows, Sustaining Members and Active Members who have paid their dues up to and including the last fiscal year shall be entitled to vote or to hold office in the Academy.

ARTICLE IV. The officers of the Academy shall be a President, as many Vice-Presidents as there are sections of the Academy, a Corresponding Secretary, a Recording Secretary, a Treasurer, a Librarian, an Editor, six elected Councilors and one additional Councilor from each allied society or association. The annual election shall be held on the third Monday in December, or on some other day in December specifically designated by vote of the Council, the officers then chosen to take office at the first meeting in January following.

There shall also be elected at the same time a Finance Committee of three.

ARTICLE V. The officers named in Article IV shall constitute a council, which shall be the executive body of the Academy with general control over its affairs, including the power to fill *ad interim* any

vacancies that may occur in the offices. Past Presidents of the Academy shall be *ex-officio* members of the Council.

ARTICLE VI. Societies organized for the study of any branch of science may become allied with The New York Academy of Sciences by consent of the Council. Members of allied societies may become Active Members of the Academy by paying the Academy's annual fee, but as members of an allied society they shall be Affiliated Members with the rights and privileges of Student Members, except the receipt of its publications. Each allied society shall have the right to delegate one of its members, who is also an Active Member of the Academy, to the Council of the Academy, and such delegate shall have all the rights and privileges of other Councilors.

ARTICLE VII. The President and Vice-Presidents shall not be eligible to more than one re-election until three years after retiring from office; the Secretaries and Treasurer shall be eligible to re-election without limitation. The President, Vice-Presidents and Secretaries shall be Fellows. The terms of office of elected Councilors shall be three years, and these officers shall be so grouped that two, at least one of whom shall be a Fellow, shall be elected and two retired each year. Councilors shall not be eligible to re-election until after the expiration of one year.

ARTICLE VIII. The election of officers shall be by ballot, and the candidates having the greatest number of votes shall be declared duly elected.

ARTICLE IX. Ten members, the majority of whom shall be Fellows, shall form a quorum at any meeting of the Academy at which business is transacted.

ARTICLE X. The Academy shall establish by-laws, and may amend them from time to time as therein provided.

ARTICLE XI. This Constitution may be amended by a vote of not less than three-fourths of the Fellows and three-fourths of the Active Members present and voting at a regular business meeting of the Academy, provided that such amendment shall be publicly submitted in writing at the preceding business meeting, and provided also that the Recording Secretary shall send a notice of the proposed amendment at least ten days before the meeting, at which a vote shall be taken, to each Fellow and Active Member entitled to vote.

BY-LAWS

AS ADOPTED, OCTOBER 6, 1902, AND AMENDED AT SUBSEQUENT TIMES

CHAPTER I

OFFICERS

1. *President.* It shall be the duty of the President to preside at the business and special meetings of the Academy and also at the meetings of the Council.

2. *Vice-Presidents.* In the absence of the President, the senior Vice-President, in order of Fellowship, shall act as the presiding officer.

3. *Corresponding Secretary.* The Corresponding Secretary shall keep a corrected list of the Honorary and Corresponding Members, their titles and addresses, and shall conduct all correspondence appertaining to such membership. He shall make a report at the Annual Meeting.

4. *Recording Secretary.* The Recording Secretary shall keep the minutes of the meetings of the Academy and of the Council; he shall have charge of all records of the Academy, and of its corporate seal, which he shall affix and attest as directed by the Council; he shall keep a corrected list of the Active Members and Fellows, and shall send to them announcements of the Meetings of the Academy; he shall notify all Members and Fellows of their election, and members of committees of their appointment; he shall notify the members of the Council of the dates of the meetings thereof; he shall lay before the Council at each meeting all matters which have come to his attention since the last meeting and which require the consideration of the Council.

5. *Treasurer.* The Treasurer shall receive all membership fees and dues, all interest accruing and paid on the invested or other funds of the Academy, and contributions to the Treasury of the Academy from any other source. All such moneys, as received, shall be deposited in Banks or Trust Companies approved and designated by the Council as depositories, in the corporate name of the Academy and subject to the drafts of the Treasurer, as such. All bills and debts against the Academy shall be paid by the Treasurer on the order of the Council for their discharge.

The Treasurer shall report to the Council at each meeting thereof a statement of the current income and expenditures, and at the Annual Meeting, he shall report to the Academy the balance sheet of the funds and the income account for the preceding year.

6. *Librarian.* The Librarian shall have charge of the library, under the general direction of the Library Committee of the Council, and

shall conduct all correspondence respecting exchanges of the Academy. He shall make a report on the condition of the library at the Annual Meeting.

7. *Editor.* The Editor shall have charge of the publications of the Academy, under the general direction of the Publication Committee of the Council. He shall make a report on the condition of the publications at the Annual Meeting.

CHAPTER II

COUNCIL

1. *Meetings.* The Council shall hold meetings at such stated times as it may decide upon, or at the call of the President. The Council shall have general charge of the affairs of the Academy.

2. *Quorum.* Five members of the Council shall constitute a quorum.

3. *Officers.* The President, Vice-Presidents and Recording Secretary of the Academy shall hold the same offices in the Council.

4. *Committees.* The Standing Committees of the Council shall be: (1) an Executive Committee consisting of the President, Treasurer, and Recording Secretary; (2) Such other committees as from time to time shall be authorized by the Council.

CHAPTER III

FINANCE COMMITTEE

The Finance Committee of the Academy shall audit the Annual Report of the Treasurer, and shall act with him in the recommendation to the Council of investments of funds of the Academy.

CHAPTER IV

ELECTIONS

1. *Sustaining, Active and Associate Members.* (a) These Members shall be nominated in writing to the Council by at least two Members who are qualified to do so by right of full membership privileges. If approved by the Council, they may be elected at the succeeding business meeting.

(b) Any Active Member who, having removed to a distance from the city of New York, shall nevertheless express a desire to retain his connection with the Academy, may be placed by vote of the Council on the list of Associate Members. Such members shall relinquish the full

privileges and obligations of Active Members, except the payment of an Associate Membership fee. They may receive the publications, in accordance with the recommendation of the council. (*Vide* Chapters V and X.)

2. *Student Members.* Junior workers in science, connected as students, junior research workers, or junior instructors with scientific institutions in or near New York City, may be elected to Student Membership for a period of five years in the manner prescribed for Active Members. They shall not have the power to vote and shall not be eligible to election as Fellows, but may receive the publications in accordance with the recommendations of the Council. At any time subsequent to their election they may assume the full privileges of Active Members by paying the dues of such Members.

3. *Fellows, Corresponding Members and Honorary Members.* Nominations for Fellows, Corresponding Members and Honorary Members may be made in writing either to the Recording Secretary or to the Council at its meeting prior to the Annual Meeting. If approved by the Council, the nominees shall then be balloted for at the Annual Meeting.

4. *Officers.* Nominations for Officers, with the exception of Vice-Presidents, may be sent in writing to the Recording Secretary, with the name of the proposer, at any time not less than thirty days before the Annual Meeting. Each section of the Academy shall nominate a candidate for Vice-President, who, on election as such, shall be Chairman of the section; the names of such nominees shall be sent to the Recording Secretary properly certified by the sectional secretaries, not less than thirty days before the Annual Meeting. The Council shall then prepare a list which shall be the regular ticket. This list shall be mailed to each Active Member and Fellow at least one week before the Annual Meeting. But any Active Member or Fellow entitled to vote shall be entitled to prepare and vote another ticket.

CHAPTER V

DUES

1. *Dues.* The annual dues of Sustaining Members shall be \$10, payable in advance at the time of the Annual Meeting.

The annual dues of Active Members shall be \$5, payable in advance at the time of the Annual Meeting.

The annual dues of Student Members shall be \$3, payable in advance at the time of the Annual Meeting.

Non-resident Associate Members shall pay an annual fee of \$3, so long as they are not entitled to the privileges of Active Membership. (*Vide* Chapter X.)

2. *Members in Arrears.* If any Member whose dues remain unpaid for more than one year, shall neglect or refuse to pay the same within three months after notification by the Treasurer, his name may be erased from the rolls by vote of the Council. Upon payment of his arrears, however, such person may be restored to Membership or Fellowship by vote of the Council.

3. *Renewal of Membership.* Any Active Member or Fellow who shall resign because of removal to a distance from the city of New York, or any Associate Member, may be restored by vote of the Council to Active Membership or Fellowship at any time upon application.

Associate Members not previously Active Members of the Academy may become Active Members, when approved by the Council, upon payment of the regular Active Membership fee.

CHAPTER VI

PATRONS, DONORS AND LIFE MEMBERS

1. *Patrons.* Any person contributing at one time \$1,000 to the general funds of the Academy shall be a Patron and, on election by the Council, shall enjoy all the privileges of an Active Member.

2. *Donors.* Any person contributing \$50 or more annually to the general funds of the Academy shall be termed a Donor and, on election by the Council, shall enjoy all the privileges of an Active Member.

3. *Life Members.* Any Member or Fellow contributing at one time \$100 to the general funds of the Academy shall be a Life Member and shall thereafter be exempt from annual dues; and any Sustaining Member who has paid annual dues for twenty-five years or more may, upon his written request, be made a Life Member and be exempt from further payment of dues.

CHAPTER VII

SECTIONS

1. *Sections.* Sections devoted to special branches of Science may be established or discontinued by the Academy on the recommendation of the Council. The present sections of the Academy are the Section of Biology, the Section of Geology and Mineralogy, the Section of Anthropology, the Section of Psychology, and the Section of Physics and Chemistry.

2. *Organization.* Each section of the Academy shall have a Chairman and a Secretary, who shall have charge of the meetings of their Section. The regular election of the Secretary shall take place at the October or November meeting of the section and the Secretary-elect shall take office on the January first following. At the time of the election of the Secretary the section shall nominate a candidate for Vice-President of the Academy, who, on election as such, shall become Chairman of the section. Vacancies in these offices shall be filled pro tempore by vote of the section; a chairman so elected being thereby nominated to the Council of the Academy for election as a Vice-President.

3. *Affiliation.* Members of scientific societies affiliated with the Academy, and members of the Scientific Alliance, or men of science introduced by members of the Academy, may attend the meetings and present papers under the general regulations of the Academy.

CHAPTER VIII

MEETINGS

1. *Business Meetings.* Business meetings of the Academy shall be held on the first Monday of each month from October to May inclusive.

2. *Sectional Meetings.* Sectional meetings shall be held on Monday evenings from October to May inclusive, and at such other times as the Council may determine. The sectional meeting shall follow the business meeting when both occur on the same evening.

3. *Annual Meeting.* The Annual Meeting shall be held on the third Monday in December, or on some other day in December specifically designated by vote of the Council.

4. *Special Meetings.* A special meeting may be called by the Council, provided one week's notice be sent to each Active Member and Fellow, stating the object of such meeting.

CHAPTER IX

ORDER OF BUSINESS

1. *Business Meetings.* The following shall be the order of procedure at business meetings:

1. Minutes of the previous business meeting.
2. Report of the Council.
3. Reports of Committees.
4. Elections.
5. Other business.

2. *Sectional Meetings.* The following shall be the order of procedure at sectional meetings:

1. Minutes of the preceding meeting of the section.
2. Presentation and discussion of papers.
3. Other scientific business.

3. *Annual Meetings.* The following shall be the order of procedure at Annual Meetings:

1. Annual reports of the Corresponding Secretary, Recording Secretary, Treasurer, Librarian, and Editor.
2. Election of Honorary Members, Corresponding Members, and Fellows.
3. Election of officers for the ensuing year.
4. Address of the retiring President.

CHAPTER X

PUBLICATIONS

1. *Publications.* The established publications of the Academy shall be the *Annals* and the *Memoirs*. They shall be issued by the Editor under the supervision of the Committee on Publications.

2. *Distribution.* One copy of the established publications shall be sent to each Benefactor, Patron, Donor, Life Member, Sustaining Member, Active Member and Fellow.

3. *Publication Fund.* Contributions may be received for the publication fund, and the income thereof shall be applied toward defraying the expenses of the scientific publications of the Academy.

CHAPTER XI

GENERAL PROVISIONS

1. *Debts.* No debts shall be incurred on behalf of the Academy, unless authorized by the Council.

2. *Bills.* All bills submitted to the Council must be certified as to correctness by the officers incurring them.

3. *Investments.* Funds in hand, arising from the maturity of existing investments or from surplus income, shall be invested by the Council in first mortgage loans or real estate or in approved negotiable securities recommended by the Finance Committee in joint action with the Treasurer.

4. *Permanent Fund.* Contributions and fees received from Benefactors, Donors and Life Members shall be credited to the Permanent Fund.

5. *Expulsion, etc.* Any Member or Fellow may be censured, suspended or expelled for violation of the Constitution or By-Laws, or for any offence deemed sufficient, by a vote of three-fourths of the Members and three-fourths of the Fellows present at any business meeting, provided such action shall have been recommended by the Council at a previous business meeting, and also, that one month's notice of such recommendation and of the offence charged shall have been given the Member accused.

6. *Changes in By-Laws.* No alteration shall be made in these By-Laws unless it shall have been submitted publicly in writing at a business meeting, shall have been entered on the Minutes with the names of the Members or Fellows proposing it, and shall be adopted by two-thirds of the Members and Fellows present and voting at a subsequent business meeting.

MEMBERSHIP
OF
THE NEW YORK ACADEMY OF SCIENCES

HONORARY MEMBERS

May 15, 1939

ELECTED

- 1912 Frank Dawson Adams, Montreal, Canada
1889 Charles Eugène Barrois, Lille, France
1937 Orpen Bower, Glasgow, Scotland
1901 Charles Vernon Boys, Andover, England
1935 Sir William Henry Bragg, London, England
1936 Henri Breuil, Paris, France
1904 Waldemar Christopher Brøgger, Oslo, Norway
1935 Robert Broom, Pretoria, South Africa
1936 Maurice Caullery, Paris, France
1937 Pierre Teilhard de Chardin, Peiping, China
1938 Edwin Grant Conklin, Philadelphia, Pennsylvania
1920 Gerhardt De Geer, Stockholm, Sweden
1936 Octave Duboscq, Paris, France
1879 Herman LeRoy Fairchild, Rochester, New York
1935 Clive Forster-Cooper, London, England
1935 S. A. Fredericksson, Stockholm, Sweden
1935 Edwin Stephen Goodrich, Oxford, England
1936 Alfred Cort Haddon, Cambridge, England
1938 Ross Granville Harrison, New Haven, Connecticut
1936 Sir Arthur William Hill, Kew, England
1935 G. L'son Horn, Stockholm, Sweden
1935 Leland O. Howard, Washington, D. C.
1936 Alex Hrdlička, Washington, D. C.
1936 Julian S. Huxley, London, England
1909 Antoine François Alfred LaCroix, Paris, France
1938 Irving Langmuir, Schenectady, New York
1936 Karl Spencer Lashley, Cambridge, Massachusetts
1935 H. Lundborg, Stockholm, Sweden
1874 Clinton Hart Merriam, Washington, D. C.
1935 John Campbell Merriam, Washington, D. C.
1935 Sir Peter Chalmers Mitchell, London, England
1936 Kingo Miyabe, Sapporo, Japan
1937 D. Obrutschew, Leningrad, Russia
1937 Charles Palache, Cambridge, Massachusetts
1900 George Howard Parker, Cambridge, Massachusetts
1898 Friedrich Carl Albrecht Penck, Berlin, Germany
1936 Charles Pérez, Paris, France
1911 Edward Bagnall Poulton, Oxford, England
1913 David Prain, Surrey, England

FLECTLD

- 1898 William Berryman Scott, Princeton, New Jersey
- 1936 Albert Charles Seward, London, England
- 1937 H. Spemann, Freiburg, Germany
- 1935 Erik A. Stensio, Stockholm, Sweden
- 1896 Joseph John Thomson, Cambridge, England
- 1935 David Meredith Searles Watson, London, England
- 1937 Franz Weidenreich, Peiping, China
- 1899 Sir Arthur Smith Woodward, Sussex, England

CORRESPONDING MEMBERS

May 15, 1939

- 1891 José G. Aguilera, Mexico City, Mexico
- 1869 Francis Ernest Engelhardt, Syracuse, New York
- 1876 John Williams Langley, Cleveland, Ohio
- 1891 Charles Riborg Mann, Washington, D. C.
- 1897 Francis Child Nicholas, New York, New York
- 1893 James Park Thomson, Brisbane, Queensland, Australia
- 1876 Leonard Waldo, New York, New York

ACTIVE MEMBERS

May 15, 1939

Fellowship is indicated by an asterisk (*) before the name

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- Anthony, R. A.
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- *Armstrong, S. T., Ph.D., M.D.
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- Bird, Henry
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- *Blumenthal, George

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- Dunscombe, George Elsworth
- Ellis, Ralph

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 Ph.D.

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JULY 13, 1939

THE MORPHOLOGY AND FUNCTIONAL EVOLUTION OF THE ATLAS-AXIS COMPLEX FROM FISH TO MAMMALS*

BY FRANCIS GAYNOR EVANS

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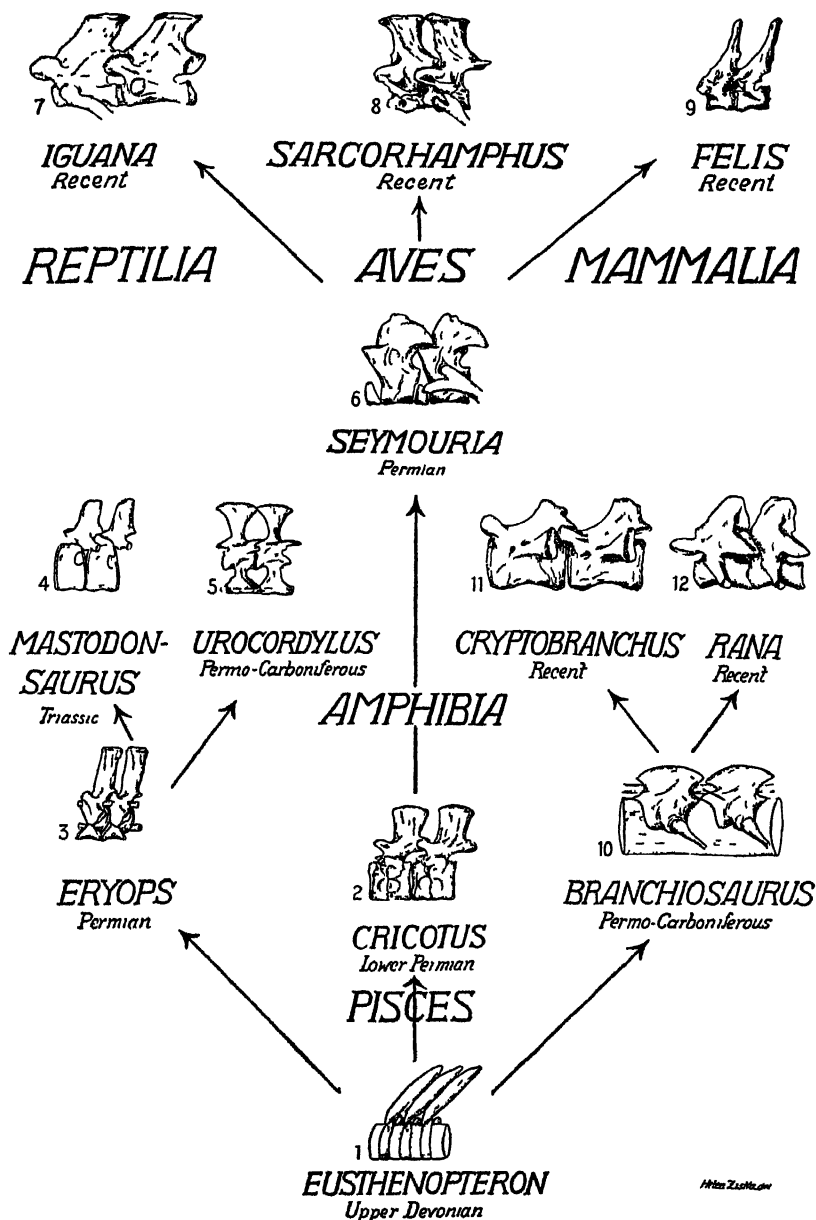
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INTRODUCTION

The literature of vertebrate morphology and palaeontology is crowded with papers on the vertebral column. Most of these, however, are purely descriptive of the vertebrae in different individual forms. A few workers, *e. g.*, Cope (1888: 243), Watson (1919: 58, 59), and Williston (1925: 90), have traced the evolution of the vertebrae in one or two different classes, but Gadow (1933) is practically the only one who has followed the evolution of the vertebrae throughout the Vertebrata. The different types of vertebrae present in various representative forms are illustrated in the form of a chart (FIGURE 1).

With respect to the muscles the situation is similar, since most investigators have been content to describe them in individual or type forms without paying much attention to their evolution. There are, however, some notable exceptions, chief among which are Adams (1919: 51), on the phylogeny of the jaw muscles of vertebrates, Huber (1930: 133, 389), on the evolution of the facial muscles, Howell (1933: 247), on the morphogenesis of the girdle and limb muscles, and Elftman (1929: 189), on the pelvic musculature of marsupials. Most of the myological work has been on the limb musculature, Vallois (1922: 1) and Nishi (1919: 692) being among the few who have worked on the axial musculature primarily. Both, however, confined their attention to recent forms, the former including fish in his work, while the latter limited himself to tetrapods, exclusive of birds.

Several authors, most of them in this country, have studied the probable condition of muscles in fossil forms and have attempted to restore them. One of the first to attempt this was Gregory, who, with Camp (1918: 450), restored the pectoral and pelvic muscles of *Cynognathus*. Gregory (1929: 703) has also restored the muscles of the Titanotheres. Later workers include Miner (1925: 145), who restored the limb muscles of *Eryops*, and Romer (1922: 519), who has done the same for the girdle and limb muscles of several fossil reptiles and amphibians. Olson (1936: 265), however, appears to be the only one who has attempted restoration of the epaxial musculature and he limited himself to recent and fossil amphibians and reptiles. Little of this



work has stressed a functional viewpoint. With the exception of Elftman's (1929: 89) work, practically the only analysis of the mechanics of bones and muscles has been made by orthopedic surgeons on the human subject and in laboratory guides for such well known animals as the cat. Some work has also been done by veterinarians on domestic animals, such as the horse and the cow.

D'Arcy Thompson (1917: 675), in his book "On Growth and Form," points out that the skeleton of an animal gives only half the picture and that, in order to have a complete understanding of how it functions in life, the associated muscles and ligaments must also be considered. It has been known for some time that the shape of bones is influenced by the attached muscles and ligaments and that shape of the former furnishes clues to the condition of the latter. It is known also that a knowledge of both the bones and their associated muscles and ligaments makes possible deductions concerning the functions of the part in question during life of the animal.

Therefore, at the suggestion of Dr. Gregory, I undertook a study of the morphology and functional evolution of the atlas-axis complex. This complex was chosen because it is the most specialized part of the vertebral column in amniotes and one on which little previous work, from an evolutionary and functional viewpoint, has been done.

MATERIALS AND METHODS

The specimens used in this study are contained in the collections of recent and fossil vertebrates in the American Museum of Natural History, New York City. The method employed was first to dissect and study the morphology and function of the atlas-axis complex in typical recent representatives of each class of vertebrates. Secondly, type fossil forms illustrating a morphological evolutionary series were selected, and a morphological and functional analysis of the complex, based on comparisons with recent representatives of each class, was made.

The types chosen for this study are: among the recent Amphibia, (1) *Cryptobranchus*, representing the Caudata, (2) *Rana*, a typical salientian; of the fossil Amphibia, (3) *Eryops*, a rhachitome, (4) a typical embolomere, (5) *Metoposaurus*, a stereospondyle; of the recent reptiles, (6) *Iguana*, a lizard, (7) *Sphenodon*, the most primitive living reptile; of the fossil reptiles, (8) *Diadectes*, a cotylosaur, (9) *Dimetrodon*, a theromorph, and (10) a cynodont; of the mammals, (11) the opossum (*Didelphis*), (12) the cat (*Felis*), and (13) man (*Homo*).

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ABBREVIATIONS

Muscles

add. mand.—adductor mandibulae
il-c. cerv. cap.—ilio-costalis cervico-capitis
intersp.—interspinalis
intertrans.—intertransversarii
levator mx.—levator maxillae
l. cerv.—longissimus cervicis
longus atlantis
longus cap.—longus capitis
longiss. cap.—longissimus capitis
obliq. cap. infer.—obliquus capitis inferior
obliq. cap. mag.—obliquus capitis magnus
obliq. cap. sup.—obliquus capitis superior
rect. cap. ant.—rectus capitis anterior
rect. cap. ant. maj.—rectus capitis anterior major
rect. cap. ant. min.—rectus capitis anterior minor

rect. cap. lat.—rectus capitis lateralis
rect. cap. post.—rectus capitis posterior
rect. cap. post. maj.—rectus capitis posterior major
rect. cap. post. min.—rectus capitis posterior minor
rect. cap. post. prof.—rectus capitis posterior profundus
rect. cap. post. superfic.—rectus capitis posterior superficialis
scalenus ant.—scalenus anterior
semisp.—semispinalis
semisp. cap.—semispinalis capitis
semisp. cerv.—semispinalis cervicis
sp.—spinalis
sp. cap.—spinalis capitis
sp. cerv.—spinalis cervicis
splenius cap.—splenius capitis
transver. cap.—transversalis capitis
trans. cerv.—transversalis cervicis

Bones

boc.—basioccipital
dso.—dermosupraoccipital
exoc.—exoccipital
fr.—frontal
ic.—intercentrum
mx.—maxilla
na.—nasal
pa.—parietal
pf.—prefrontal

plc.—pleurocentrum
pmx.—premaxilla
ptg.—pterygoid
pto.—prootic
qu.—quadrate
soc.—supraoccipital
sq.—squamosal
tab.—tabular
tym.—tympanic

MORPHOLOGY AND FUNCTION OF THE ATLAS-AXIS COMPLEX IN FISH

The cervical region or neck of an animal contains no extension of the coelom and typically is that part between the skull and the pectoral girdle. It first appears in tetrapods, and in typical sub-tetrapods there is no specialization of either the vertebrae or the muscles in this region to differentiate it as a neck.

While we are concerned chiefly with the modifications of the first two vertebrae, as we go up the vertebrate scale the muscles must be considered in order to understand how these vertebrae functioned in life. Of course only the bones are preserved in fossil forms, but their size and shape and a knowledge of the conditions in modern forms give a good idea of what the neck muscles were probably like.

In recent forms the cervical muscles belong to the axial group extending along the dorso-lateral surface on either side of the vertebral column. They are voluntary, striated, segmental muscles that arise from the metameric somites of the embryo. In the lower vertebrates they are divided by a horizontal septum into an upper (epaxial) and a lower (hypaxial) portion, and extend without interruption from the occiput to the end of the tail.

Throughout the vertebrates, these muscles are innervated by the spinal nerves, the epaxial division being supplied by the dorsal ramus and the hypaxial division by the ventral ramus of the nerve for that segment. Consequently, the question of muscle homology based on innervation is not such a problem in dealing with these muscles as it is with others, since a particular muscle can be moved a few segments up or down the axis of an animal without changing its homology.

Petromyzon (FIGURE 2, A), one of the most primitive living vertebrates, shows the primitive condition of the trunk muscles. Here there are no epaxial and hypaxial divisions, but the entire muscle mass is divided by septa into a number of myomeres. These myomeres correspond in number to the vertebral elements and extend uninterruptedly throughout the length of the animal. The septa, only slightly bent, give the body the appearance of being composed of a series of muscle bands or hoops. The parallel muscle fibers extend horizontally between successive septa which are attached to the insignificant cartilaginous neural arch rods, since no centra or ribs are present. The animal swims by wriggling movements of the body produced by alternate waves of contraction in the axial muscles.

In the primitive gnathostomes, represented by *Squalus* (FIGURE 2, B),

several advances over the cyclostome condition are noted. The most important is the appearance of a well developed horizontal septum dividing the body musculature into epaxial and hypaxial regions.

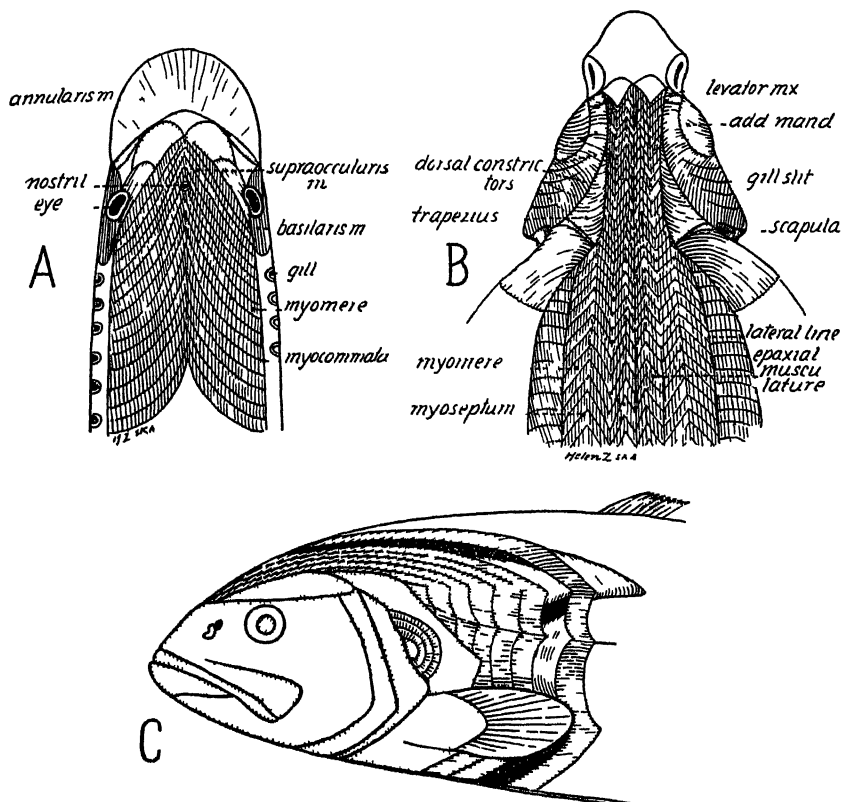


FIGURE 2 Epaxial musculature in sub-tetrapods A *Petromyzon* B *Squalus* C *Myxotropera*

The distinctly segmented epaxial portion is composed of a series of myomeres extending from the occiput to the base of the caudal fin. In the anterior part of the body it is slightly interrupted by the scapula and is beginning to be crowded and overlapped by the gill arch and pectoral musculature.

The septa have changed direction and instead of being more or less simple vertical partitions they are now folded into W's and are attached to the cartilaginous neural arches, supradorsals, centra and ribs. The muscle fibers continue to run almost horizontally between the septa. This folding of the septa produces a series of cones within

cones, so that in a cross section of the body several septa and myomeres are visible. The W-shaped arrangement of the septa is of fundamental importance for locomotion and enables the shark to be a fast, active swimmer. As Greene and Greene (1914: 30) have shown in the King Salmon, this arrangement gives the septum the effect of a tendon, so that the force of a contraction of a myomere is distributed over several segments. The folded arrangement puts the muscle fibers in the best mechanical position to expend their force of contraction in a more direct pull on the septum and axial skeleton than would be possible if the septum were a simple vertical plate at right angles to the axis of the body.

These modifications are carried to even greater extent in the bony fishes, where the septa become more sharply folded and the myomeres overlap each other more extensively than in the elasmobranchs. By this time there are well ossified centra, neural arches and spines, and ribs to serve as bases for attachment of the septa. There is still no definitive neck and the segmental trunk muscles are attached to the back of the skull without a break in their continuity. In *Mycteroperca* (FIGURE 2, C), these muscles extend over the top of the skull to a position between the eyes. In the gill region the epaxial musculature is reduced in volume by the presence of the branchiomic muscles.

The vertebrae in bony fish generally consist of a well developed bony centrum, the ossification of which extends over the neural and haemal arches so that they are continuous with it. These vertebrae are usually amphicoelous and strongly constrict the notochord. Typically, there is nothing approaching an atlas-axis complex and the first two vertebrae usually differ from those behind them only in being a little shorter.

According to Ford (1937: 5), the anterior end of the spine in bony fish is modified to perform the functions taken over by the atlas-axis in the higher vertebrates. There is much specific variation but normally these functions—attachment of the skull to the spinal column and provision of an axis on which the head moves—are performed by the first few vertebrae or sometimes by just the first one.

The height of the spinous processes varies in different fish but in typical bony fish there are long, curved pleural ribs. All this is correlated with the great mass of the axial musculature which, as noted above, is well developed and is the chief locomotor organ of the animal.

In the active, free-swimming forms the skull is usually high and rather narrow, whereas in the less active, bottom-living forms it tends to be broad and flattened.

MORPHOLOGY AND FUNCTION OF THE ATLAS-AXIS COMPLEX IN LOWER TETRAPODS

Amphibia

The rise of the tetrapods is associated with the appearance of a definitive neck and of an atlas-axis complex, both of which are absent in fish. The appearance of the neck was caused by the separation of the pectoral girdle from the skull and its subsequent posterior migration. This occurred independently in the sharks without any special modification of either the vertebral column or the axial muscles, to differentiate this region as a neck.

The atlas-axis arose as an adaptation to permit greater mobility of the head on the spinal column and first appeared as a simple structure quite different from the specialized mechanism seen in mammals.

CRYPTOBRANCHUS

Since an understanding of the atlas-axis complex of recent forms is a prerequisite to a comparative study of the complex in fossil forms, the present survey may be begun with the atlas-axis of *Cryptobranchus*, a modern representative of the Amphibia, the oldest and most primitive class of tetrapods.

Cryptobranchus (FIGURE 3, B) has a broad flat skull with two well developed exoccipital condyles, which are quite widely separated as there is no basoccipital bone between them. The broad articular surfaces of the condyles are flat ovals that face slightly inward. Between them and beneath the foramen magnum is a well marked pit which receives the odontoid process of the first vertebra.

The vertebrae, which have also shared in this flattening process, possess broad neural arches with truncate spines which are triangular in dorsal view. The well developed transverse processes articulate with short rod-like ribs. The neural arches are fused with the centra.

The neck is very short and there is only one true cervical vertebra (FIGURE 3, A). This first vertebra, which serves the purpose of the first two in amniotes, has on the cranial surface two articular facets with an odontoid process between them. The facets are broad flat ovals articulating with the occipital condyles, while, as noted above, the odontoid process fits into the depression below the foramen magnum. The spinous process is shorter and broader antero-posteriorly than that of the succeeding vertebrae, and there are well developed postzygapophyses set at a small angle above the horizontal. The atlas

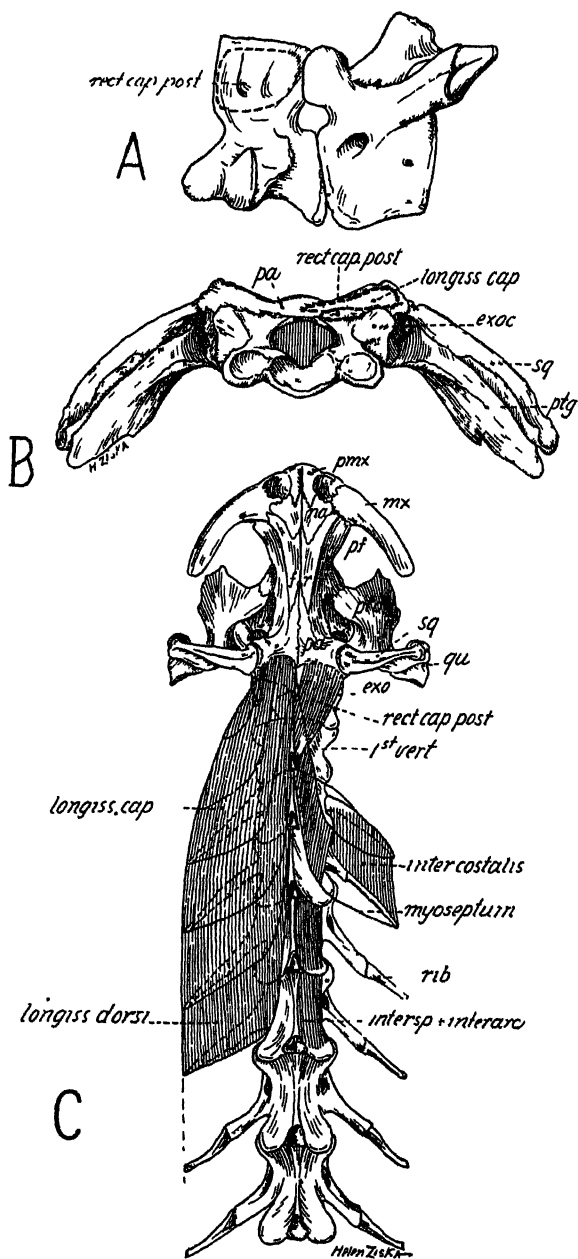


FIGURE 3. *Cryptobranchus*. A. First two vertebrae, showing origins of occipital muscles. B. Occipital view of skull, showing insertions of epaxial muscles. C. Epaxial muscles.

differs from all the other presacral vertebrae in lacking transverse processes and ribs.

The second vertebra is very similar to the succeeding presacral vertebrae and like them bears a truncate spinous process, well developed zygapophyses set at a small angle above the horizontal, and short distally expanded transverse processes articulating with short rod-like ribs. Like the first vertebra, it is a little shorter than the other presacral vertebrae.

The occipito-vertebral joint described above allows flexion and extension of the head on the spinal column. There is also rotation about the odontoid process but practically no lateral movement. The range of movement is limited by the joint capsules, by the spinal ligaments, and by the action of antagonistic muscles.

The articulations of the second vertebra with the first and third allow lateral movements but no flexion, extension, or rotation.

Here we have a suggestion of that division of labor in the first two vertebrae which is carried to its extreme stage in mammals. Nevertheless, the atlas of *Cryptobranchus* is not as specialized as that of the higher amniotes, since it is not ring-shaped and does not rotate about the second vertebra. Consequently, the rotation of the head occurs at the joint between the skull and the first vertebra, whereas in the higher amniotes it occurs at the joint between the first and second vertebrae.

The simple, dedifferentiated condition of the vertebral column and ribs to form a string of secondary polyisomeres is reflected in the simplified epaxial musculature (FIGURE 3, C), which consists of a series of myomeres extending from the head to the end of the tail. Since *Cryptobranchus* is a sluggish form, these myomeres are band-like and not highly folded as in an active fish.

The general epaxial muscle mass, the sacro-spinalis, is not differentiated into longitudinal muscles and is entirely metameric. The muscle fibers extending between successive neural spines and transverse processes have been designated as the interspinous and the intertransversarii, respectively. The trunk musculature extends uninterruptedly into the neck region and is inserted on the occiput as the longissimus capitis. The deeper fibers, running obliquely from the first spinous process to the occiput, form the rectus capitis posterior.

The almost horizontal position of the zygapophyses throughout the vertebral column allows good lateral movement but extremely little, if any, flexion and extension and no rotation.

The sacro-spinalis muscles bend the body laterally while the longis-

simus capitis and the rectus capitis posterior extend and rotate the head. The head can also be turned to the side by bending the whole anterior end of the body.

The action of the epaxial muscles is opposed by that of the subvertebral musculature, which runs along the ventral surface of the spinal column from the head to the tail. These muscles are inserted on the base of the skull and arise from the centra, those of the opposite sides, in the latter case, being separated by a median ventral keel. These muscles are the flexors of the spinal column, and the part inserted on the skull flexes the head.

RANA

The skull of the frog is also very flat and is even more fenestrated than that of *Cryptobranchus*. In occipital view (FIGURE 4, B) the region above the foramen magnum slopes forward and the occipital condyles are well developed and widely separated. The condyles are formed from the exoccipital bones and have convex articular surfaces.

There is only one true cervical vertebra (FIGURE 4, A), which differs from the succeeding ones in lacking the transverse processes and in having on the cranial surface two concave articular facets for the occipital condyles. The odontoid process between these facets is reduced in size and the entire vertebra is much wider cranially than caudally. The spinous process is low but longitudinally elongated so that it is much longer than that of any succeeding vertebra. Prezygapophyses are absent but obliquely placed postzygapophyses are present.

This type of occipito-vertebral joint allows flexion, extension, and some lateral and rotational movement. None of these movements, however, has a very great range. Flexion is restricted by the cranio-ventral projection from the atlas, while the widely separated, convex occipital condyles allow only slight lateral and rotational movements. Extension is limited by contact of the skull with the atlas.

The Salientia have apparently always been more specialized than the Caudata, as is shown by the vertebral column which has only nine free vertebrae, the smallest number in any known vertebrate. As a result of this extreme shortness, movements of the spinal column are very limited. The zygapophyses are flat and practically horizontal in position. The transverse processes are long straight rods, which in some species represent a fusion with the ribs.

This more specialized condition of the spinal column is reflected in the epaxial musculature, which is also more highly differentiated than in *Cryptobranchus*. Here it shows a tendency to be divided into a

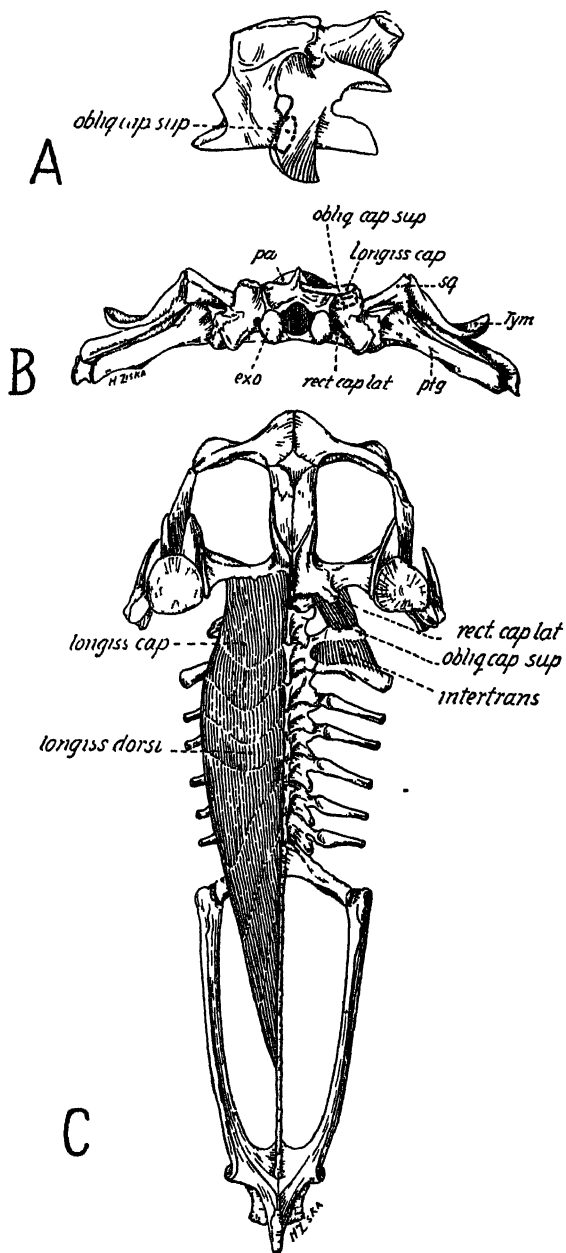


FIGURE 4 *Rana* A. First two vertebrae, showing origins of occipital muscles B. Occipital view of skull, showing insertions of occipital muscles C. Epaxial muscles

longitudinal lateral mass, the ilio-lumbaris, and a medial part, the longissimus dorsi (FIGURE 1, C'). The epaxial musculature is divided by septa into the same number of myomeres as there are vertebrae, but the myomeres are not all similar as they are in *Cryptobranchus*.

In the cervical region the muscles are more specialized than in *Cryptobranchus*, in that an obliquus capitis superior and a rectus capitis lateralis are present. These muscles run between the first transverse process and the occiput and are in series with the intertransversarii muscles. The obliquus capitis superior (FIGURE 4, B) is inserted on the fronto-parietal; the rectus capitis lateralis, on the ventral surface of the exoccipital.

Some flexion and extension is possible in the anterior part of the column but all movements are very slight.

ERYOPS

The condition of the atlas-axis in the fossil Amphibia may now be considered. The primitive embolomeeres, which first appeared in the Devonian, probably had an atlas-axis but it has not been found. However, a sound basis for its restoration is afforded by the complex in the rhachitomous Amphibia of the Permian, their direct descendants. One of the best known genera of these Permian rhachitomes is *Eryops* (FIGURE 5), of which the American Museum has several specimens of the skull and one of the anterior part of the vertebral column.

Comparison of the occipital region of the skull of *Eryops* (FIGURE 5, C) with that of *Cryptobranchus* (FIGURE 3) shows that fundamentally the two are very similar, although *Eryops* shows only the initial stages in flattening of the head. In both, the supraoccipital region slopes forward, much more so in *Cryptobranchus* than in *Eryops*, and both have large oval exoccipital condyles with flat articular surfaces facing downward and inward. *Eryops*, however, retains the basioccipital bone as a small ventro-median part of the condyle, which is actually tripartite. The basioccipital bone has been lost in the modern amphibians. Both forms have a depression below the foramen magnum but it is quite shallow in *Eryops*. The retention in *Eryops* of an ossified basioccipital may be, according to Gadow (1933: 67), correlated with the retention of the hypoglossal as a cranial nerve, a condition not found in modern amphibians.

The neural arch of the atlas in *Eryops* is well developed and has a bifid spinous process which is inclined backward so as to grasp the neural spine of the axis between its forks (FIGURE 5, A). On the cranial surface of the arch are two little nubbins of bone, one on each side,

which represent the remains of the proatlas. This was probably better developed than in any living forms (*e. g.*, *Sphenodon* and crocodiles), and articulated with two small facets on either side of the foramen

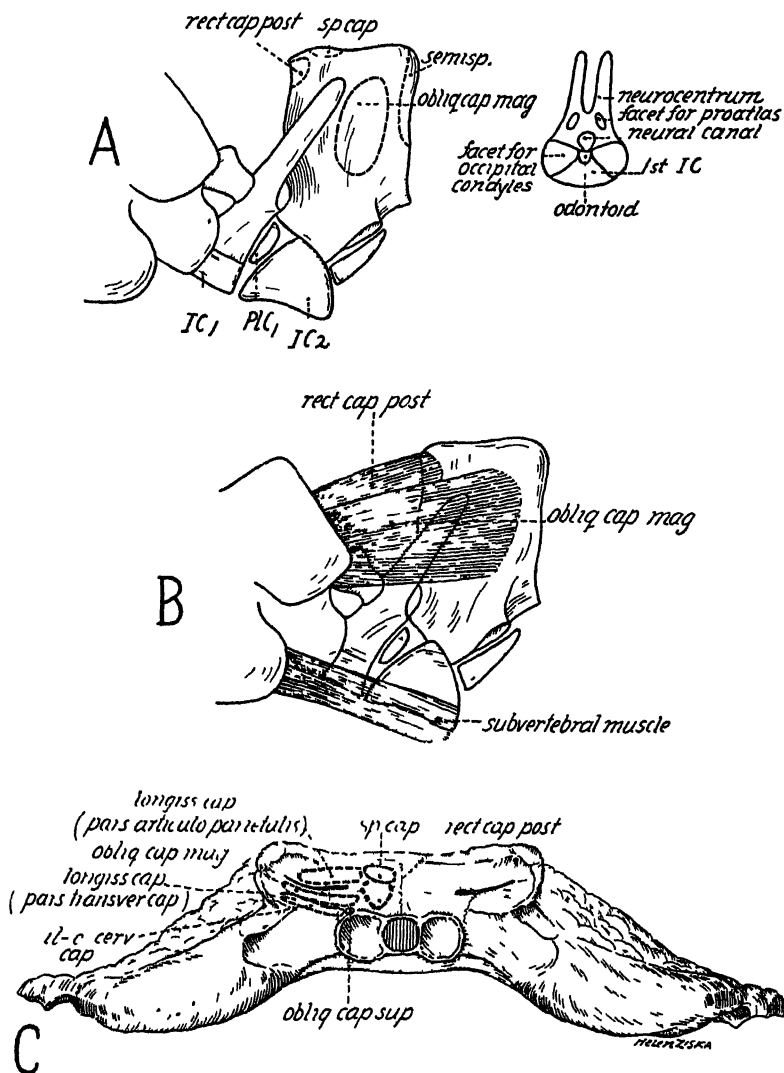


FIGURE 5 *Eryops* A. Atlas-axis, showing origins of occipital muscles and front view of atlas B Restoration of occipital and subvertebral muscles C. Occipital view of skull, showing insertions of epaxial, occipital and cervical muscles. Modified from Olson (semi-diagrammatic).

magnum. According to Goodrich (1930: 68), a proatlas has also been recorded in many Cotylosauria, Theromorpha, Rhynchocephalia, and Dinosauria. The above is the usual type of rhachitinous atlas neural arch. A similar condition has been described in *Trimerorhachis* and *Trematops* by Case (1911: 108, 134), and in *Cacops* by Williston (1910: 263).

The centrum of the atlas (first intercentrum) is not preserved in the museum specimen but it has been figured in place by Branson (1905: 607, fig. 19) as crescentic in shape and fairly large, although smaller than the second intercentrum. It was not fused with the neural arch of the atlas as in *Cacops* but was doubtless firmly sutured to it, inasmuch as the heavy, massive head would require a strong, firmly united first vertebra for articulation. The cranial surface must have had large flat oval facets for articulation with the occipital condyles. This centrum was probably similar to that of *Cryptobranchus*, since the occiputs of the two are so similar, and probably, like it, had an odontoid process that fitted into the depression below the foramen magnum. In *Eryops* this process would have articulated with the basioccipital bone.

The bases of the neural arches of the atlas and the axis are quite close together so that the pleurocentrum, if present, must have been small.

The axis shows the typical rhachitinous components and is characterized chiefly by its expanded spinous process, which is wedged between the forks of the neural spine of the atlas. The transverse processes, in contrast to those of the atlas, are well developed and articulate with single-headed ribs. The postzygapophyses are well developed but the prezygapophyses are small, to coincide with postzygapophyses of the atlas.

Functionally *Eryops* was practically bicondylic. The widely separated, flat exoccipital condyles prevented lateral movement of the head on the vertebral column. Some rotation of the head about the odontoid process, similar to that occurring in *Cryptobranchus*, may have been possible, depending on how tightly the proatlas articulated with the skull and the atlas. Flexion and extension were also possible at the occipito-vertebral joint. The range of extension would depend on the proatlas articulation and the elasticity of the joint capsules, but in any case it would be quite limited since the proatlas would act as a wedge or brace between the occiput and the atlas. The range of flexion would also be limited by the closeness of the proatlas articulation with the skull and the atlas and by the elasticity of the joint capsules and membranes between the skull and the atlas.

The entire occipito-vertebral joint was quite stiff and closely articulated to support the heavy head. The neck was short, containing only three or four vertebrae, and the head was probably moved chiefly by the cervical muscles acting on the entire neck.

Lateral movement or rotation at the atlanto-axial joint was practically impossible in *Eryops*, because the neural spine of the axis is firmly held between the forks of the spinous process of the atlas. There may have been a very slight amount of flexion and extension at this joint, depending on how tightly the spinous process of the atlas clasped that of the axis.

The postzygapophyses of the axis are at about a 40° angle from the horizontal, which would allow lateral motion, rotation about an oblique axis, and some flexion and extension at the joint between the axis and the third cervical vertebra. Extension could not have been very great due to the close proximity of the neural spines of these vertebrae, which would soon touch each other.

The above movements were produced by the action of the occipital and cervical muscles, which have been restored by Olson (1936: 273, 285, 299) and are summarized, according to function, in the following table.

TABLE I
OCCIPITAL AND CERVICAL MUSCLES IN PRIMITIVE AMPHIBIA
Extension of Head and Neck

MUSCLE	ORIGIN	INSERTION
Rect. Cap. Post.	Dorso-cranial extremity of the spinous process of the axis	Supraoccipital region of the skull
Sp. & Semisp. Cap.	Spinous processes of all the cervicals except the first	Dermosupraoccipital
Sp. & Semisp. Cerv.	Fleshy from the Sp. dors.	Spinous processes of the 2nd and 3rd vertebrae

Rotation and Lateral Turning of Head and Neck

MUSCLE	ORIGIN	INSERTION
Longiss. Cerv. & Cap.	Longiss. dorsi and cervical ribs	Occiput and atlas
Ilio-cost. Cerv. & Cap.	Ilio-cost. dorsi	Cervical ribs and opisthotic

An interspinatus and a supraspinatus ligament were probably present, as in most modern forms, and undoubtedly aided greatly in supporting the head and spine. Movements of the head were produced by the occipital muscles (FIGURE 5, B). The ventro-lateral

surfaces of the intercentra are concave and have a fairly distinct median keel, indicating the presence of a well developed subvertebral musculature. This musculature, as in modern salamanders, probably ran the length of the vertebral column and was inserted on the intercentra and on the ventral surface of the transverse processes and the occiput. These muscles were the flexors of the spinal column and head, opposing the action of the dorsal extensors.

EMBOLOMERI

Our knowledge of the morphology and function of the atlas-axis in *Eryops* furnishes a good working basis for analysis of the complex in the primitive ancestral amphibian, which was probably an embolomere or closely related type.

Watson (1926: 189) has described and figured the skull of several embolomeres which show the condition in the most primitive known Amphibia. Here the skull, instead of being flattened as in *Eryops* and tending toward the extreme flat type of the modern amphibians, is high and almost rectangular in occipital view. The occiput was more primitive than in *Eryops*, in view of the large, well ossified basioccipital bone bearing a single well developed condyle. The latter was circular with a concave articular face and the exoccipital bones took little part in its formation. These articulated with the supraoccipital, and in *Orthosaurus* their caudal surface had an articular facet borne on a low projection. This facet probably articulated with a proatlas, since it occupies about the same position as the one in *Eryops* that is supposed to have articulated with the proatlas. The hypoglossal nerve was included within the skull and emerged through a foramen in the exoccipital.

Unfortunately the first few vertebrae of these forms have not been well enough preserved to disclose much of their structure. However, from what is known about the atlas-axis of *Eryops* and other rhachitomes, the primitive embolomere atlas-axis must have been somewhat as follows: the atlas neural arch (FIGURE 6) was probably less inclined posteriorly than in *Eryops* and was only slightly forked, so that it just grasped the cranial edge of the spinous process of the axis. Its cranial surface probably articulated with a proatlas as in *Eryops* and *Sphenodon*.

The presence of a proatlas in *Sphenodon*, the most primitive living reptile, is doubtless an ancient character inherited from its amphibian ancestors. This structure is found in the primitive theromorph reptiles and, as already noted, was probably present also in the

rhachitinous amphibians. All this evidence therefore indicates its probable presence in the primitive ancestral embolomere.

Below, as in other regions of the embolomere vertebral column, the neural arch articulated with both the anterior intercentrum and the posterior pleurocentrum. The latter, as figured by Watson (1926: 228) in *Eogyrinus*, was probably the larger and formed the chief support for the neural arch of the atlas. These centra were notochordal and amphicoelous. The cranial face of the first intercentrum probably had an articular surface for the occipital condyle.

The axis was probably little, if any, different from the succeeding vertebrae and showed only a slightly expanded neural spine. There

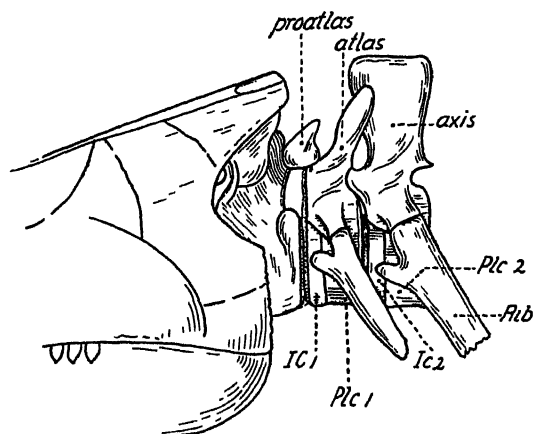


FIGURE 6. Restoration of the atlas-axis complex in a primitive ancestral embolomereous amphibian.

was probably not the great discrepancy in size between the prezygapophyses and postzygapophyses that was noted in *Eryops*. This may be due to the fact that the atlas was not yet very specialized and still possessed normal-sized zygapophyses. The transverse processes of both atlas and axis bore ribs, a more primitive feature than in *Eryops*.

The atlas-axis of these earliest tetrapods, as seen above, differed little from the rest of the vertebrae and was not specialized for complicated movements of the head. In all probability the movements at the occipito-atlantar joint were little, if any, greater than those between adjacent vertebrae. The single concave occipital condyle and the articulation of the proatlas with the skull and atlas would allow a small amount of lateral motion and rotation but very little flexion or extension. The latter movement was most limited by the

close proximity of the occiput and the proatlas, which would come in contact after a very short extension of the head. The articulation between the proatlas and the prezygapophyses of the atlas probably allowed a slight lateral motion but no flexion or extension. The atlantar postzygapophyses were doubtless normal and, as recorded by Watson (1926: 229) for the dorsal region of *Eogyrinus*, at about a 45° angle above the horizontal. However, the grasping of the spinous process of the axis by that of the atlas must have prevented practically all movement between these vertebrae.

The head and the first two vertebrae in embolomeres seemingly moved as a more or less single piece, with most of the motion in this region occurring between the axis and the third vertebra. The neck of these early forms, due to the close proximity of the shoulder girdle to the skull, was short and for the same reason not very mobile.

It may be assumed that the neck muscles were very similar to those of *Cryptobranchus* and represented a cervical extension of the trunk musculature, as indicated by the moderately well developed spinous processes and ribs which are little different from those in the trunk. The muscle fibers running between the atlas and the occiput may have been slightly separated from the general muscle mass to form a rectus capitis posterior as in *Cryptobranchus*. These muscles would turn the head and neck laterally and also extend them slightly.

To offset the action of these muscles there must have been, as in modern amphibians, a subvertebral muscle running just beneath the vertebral column. As in modern forms, this muscle probably arose from the centra and was inserted on the ventral side of the occiput. According to Watson (1926: 208) *Orthosaurus* shows the first traces of the basisphenoid tubercle for the recti capitis muscles. These muscles flexed the head and neck on the spinal column. The range of movement was limited by the antagonistic action of the dorsal extensor muscles and also by the supraspinus and interspinatus ligaments, as in all tetrapods.

The presence of long curved ribs indicates that the transversalis muscle may also have been present. If present, it would aid the epaxial muscles in bending the vertebral column, and indirectly the head, to one side.

These earliest amphibians were still very fish-like in many respects. One of them, according to Watson (1926: 234), even retained the fish characteristic of having the pectoral girdle attached to the skull by a posttemporal bone.

The spinal column of these forms showed slight regional differentia-

tion into a presacral and a caudal region, with long curved ribs on all the presacral vertebrae. The moderately developed spinous processes showed little difference in height in various parts of the body. This feature, together with the long curved ribs, suggests that the epaxial musculature was quite well developed. Doubtless it was still the chief locomotor organ, inasmuch as the legs were weak and the animal must have wriggled its way over the surface of the ground by alternate contractions of the axial muscles in the same way that a fish swims. Watson (1926: 202) states that "this motion by itself will enable the animal to walk without any independent movement of the limb apart from such motion in a vertical plane as is necessary to enable it to engage and to be lifted from the ground . . ."

The epaxial musculature in these early forms presumably consisted of a series of myotomes extending from the occiput to the end of the tail. In the sacral region it was probably interrupted slightly by the pelvic girdle and in the pectoral region overlapped to some extent by the shoulder muscles. The moderately developed neural spines, long curved ribs giving the body a rounded contour, and the high skull resemble the condition in an active, free-swimming fish more than they do the flattened skull and spinal column and short, rod-like ribs of a sluggish, bottom-living form like *Cryptobranchus*. Consequently the epaxial musculature, as indicated by the skeleton and in keeping with the supposedly more active habits, was probably of greater volume and the myomeres showed more folding than in a modern urodele.

The above represents the primitive tetrapod condition of the epaxial musculature, the later modifications of which have been worked out for fossil forms by Olson (1936: 265) and in recent forms by Nishi (1919: 292), Vallois (1922: 1), and others.

Primitively, as seen above, a cervical segment consisted of a vertebra with a moderately developed neural spine and transverse processes, well developed curved ribs, and an unmodified cervical extension of the dorsal trunk musculature. It was primarily locomotor in function, the same as the trunk segments, and was little, if any, different from them. The changes occurring during the course of evolution will be pointed out later.

STEREOSPONDYLI

In the stereospondylous amphibians, which Watson (1919: 50) has shown to be direct descendants of the Rhachitomi, the tendency toward a flattened skull initiated in *Eryops* is continued to such an extent that the skull in some genera (e. g., *Plagiosternum*), becomes

very broad and flat. The same thing is seen in the latest lepospondyles, such as *Diplocaulus*; this is an excellent example of parallelism, perhaps to be correlated with the similar bottom-living habits of the two groups.

The tendency, first seen in *Eryops*, toward the development of a double condyle by reduction of the basioccipital bone and emphasis of the exoccipital bone, is carried still further. In some of the Upper Triassic stereospondyles the basioccipital is greatly reduced and fails to become ossified.

In occipital view the skull of *Mastodonsaurus*, as figured by Fraas (1889: 69, *fig. 2*), is broad and flat, with two well developed, widely separated, exoccipital condyles, the basioccipital having disappeared. The condyles have oval convex faces for articulation with the atlas, which Fraas (1889: 77, *pl. 3, fig. 2, 3*) has also described and figured. The latter has a disc-shaped centrum bearing a low neural arch and spine and, on its anterior surface, two large oval concave facets for articulation with the occipital condyles. The atlas, with a concave posterior surface, is amphicoelous like the rest of the vertebrae. There is no indication of a proatlas, transverse processes, or ribs. An occipito-vertebral joint of this type allows flexion, extension, and a small amount of lateral movement combined with rotation.

The axis differs from the atlas only in lacking the two articular facets on the cranial surface of the centrum and in possessing transverse processes and ribs. The succeeding vertebrae are similar. The zygapophyses of the latter are moderately developed and in Fraas' figures appear to be at a slight angle above the horizontal. This would permit lateral movement and oblique rotation but little flexion or extension.

The flattening of the skull is associated with a reduction in height of the spinous processes of the vertebrae, which in turn indicates a decrease in size of the epaxial musculature.

The vertebral column of one of these forms, *Metoposaurus* (*Metopias*), as figured by Fraas (1889: *pl. 14*), shows a striking resemblance to that of *Cryptobranchus*. In both the vertebrae are wide and bear truncate spinous processes. In *Cryptobranchus* the vertebrae are longer than in *Metoposaurus*, in which the closely adjacent spinous processes reduce the range of extension in the vertebral column. In both, well developed exoccipital condyles extend quite a distance behind the skull. The ribs of *Metoposaurus* are shorter and straighter than in *Eryops* and approach the condition in *Cryptobranchus*, although showing less reduction.

This dedifferentiation of the spinal column, as in *Cryptobranchus*, is

probably a secondary character correlated with sluggish, bottom-living habits, which are indicated by the shape of the head and body. The reduction in size and length of the ribs is also consistent with such habits, since long curved ribs are not needed to protect the viscera, which are no longer subjected to the violent contractions of the axial muscles characteristic of an active form such as a fish.

The flat skull, short spinous processes, and ribs indicate that the epaxial musculature was reduced in volume. Also, it seems likely that this musculature was less differentiated than in *Eryops*, a more typical terrestrial form, and more nearly resembled that in *Cryptobranchus*, as indicated by similarities between the vertebral column, ribs, and skull in the two forms.

Reptilia

SPHENODON

An analysis of the atlas-axis in fossil reptiles requires the same procedure as used with the Amphibia—i. e., a study of its condition in some modern form as a solid basis for understanding the complex in fossils. The modern forms chosen for this purpose are *Sphenodon*, the most primitive living reptile, and *Iguana*, a typical lizard.

In *Sphenodon* the skull is more highly fenestrated than in the fossil amphibians but is less specialized, with respect to reduction in the number of elements, than that of modern Amphibia. In occipital view (FIGURE 7, C) it is quite high and, except for the large post-temporal fenestra, resembles the skull of the embolomeres more than that of modern amphibians. There is a single oval basioccipital condyle with a convex articular surface. The exoccipital bones are proportionately much less developed than in the early amphibians and contribute only a small portion to the condyle, a condition quite different from that in all Amphibia above the Rhachitomi.

The atlas is more specialized than in any amphibian in view of the loss of its centrum, which is fused with that of the axis to form the odontoid process. The atlas neural arches, together with the first intercentrum, tend to form a ring that rotates about the odontoid. The cranial surface of the first intercentrum has a concave facet for articulation with the occipital condyle, while the caudal surface is convex dorso-ventrally to articulate with the horizontal groove on the odontoid process. The neural arches of the atlas are rather small, spineless, and separate above the spinal cord. They articulate with the neural spine of the axis posteriorly and with the proatlas anteriorly. The latter is a small triangular ossicle, one on each side, that articulates with the skull anteriorly and with the neural arch of the atlas

posteriorly. At the base of the atlas neural arch is a small facet for the occipital condyle and at the back is another for the odontoid.

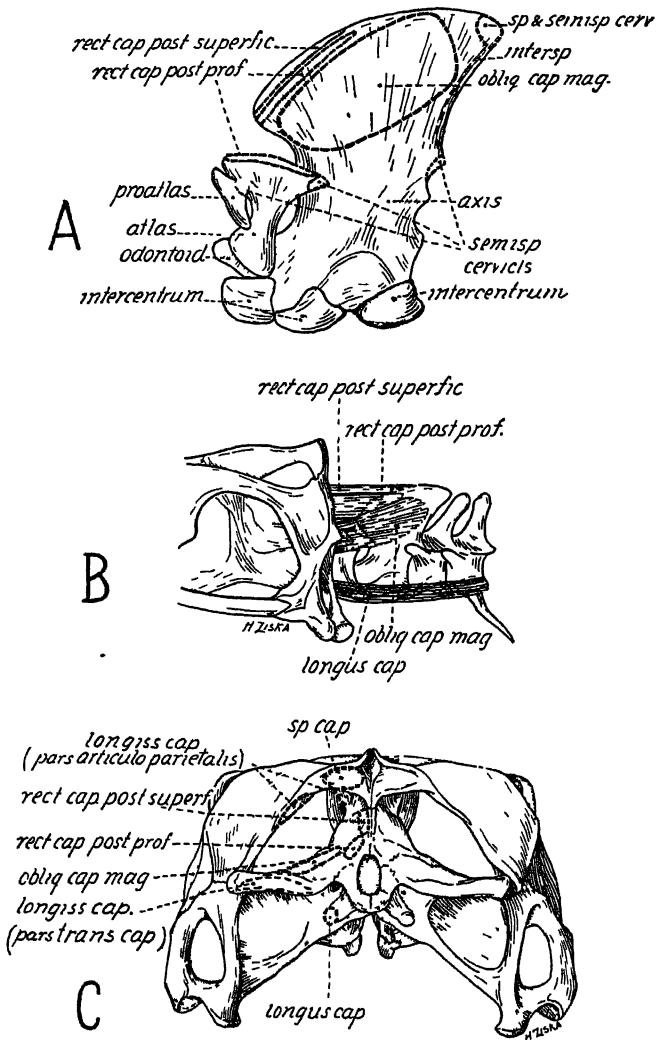


FIGURE 7. *Sphenodon*. A. Atlas-axis complex, showing origins of occipital muscles. B. Suboccipital and subvertebral muscles. C. Occipital view of skull, showing insertions of occipital and cervical muscles (semi-diagrammatic).

Small postzygapophyses are present but no transverse processes or ribs.

The axis (FIGURE 7, A) is characterized by a large, hatchet-shaped

spinous process, different from that in the amphibians, which articulates with the atlas neural arch anteriorly and with the third cervical vertebra posteriorly. The well developed prezygapophyses and postzygapophyses are set at a slight angle above the horizontal. The neural arch and centrum are fused and there are no transverse processes or ribs. Fused to the cranial end of the centrum of the axis is the odontoid process, morphologically the centrum of the atlas, and below it is the intercentrum of the axis. These two together form a saddle joint whose articular surface is concave dorso-ventrally and convex from side to side. This surface articulates with the atlas.

This joint permits good lateral motion and rotation but flexion and extension are more limited. In these movements the atlas and head move as one piece. Rotation occurs between the atlas and the axis in higher amniotes, instead of between the skull and the first vertebra as in Amphibia.

The movements occurring at the occipito-vertebral joint are not very great. The convex articular surface allows flexion, extension, and a little rotation, but not lateral bending. All these movements have approximately equal ranges and depend largely on how tightly the proatlas articulates with the skull and on the elasticity of the joint capsules and vertebral ligaments.

These movements are produced by the action of the occipital and cervical muscles (FIGURE 7, B), which are more specialized than in the Amphibia. They are summarized according to action in TABLE 2. The subvertebral muscles are shown in FIGURE 15, A.

IGUANA

The other modern reptile to be considered is *Iguana*, a typical lizard. The skull in occipital view (FIGURE 8, C) is quite similar to that of *Sphenodon* except that it is more fenestrated and a little higher and narrower. There is a single convex occipital condyle, which is crescentic in shape and has a shallow groove on its dorso-caudal surface.

The atlas (FIGURE 8, A) is a ring which is open dorsally and has a well developed hypapophysis. On the ventral half of the cranial surface of the ring is a concave articular surface for the occipital condyle. This facet is divided into two small lateral surfaces which articulate with similar surfaces on the dorso-lateral surface of the condyle, and a larger ventro-median surface which articulates with a corresponding part of the condyle. The neural arches of the atlas are short and do not meet over the spinal cord. There is no spinous process nor are there prezygapophyses, but tiny postzygapophyses are

TABLE 2

OCCIPITAL AND CERVICAL MUSCLES IN REPTILES

Extension of Head and Neck

MUSCLE	ORIGIN	INSERTION
Rect. Cap. Post. Superfic.	Dorso-cranial border of neural spine of axis	Occipital Crest (<i>Sphenodon</i>) Supraoccipital (<i>Iguana</i>)
Rect. Cap. Post. Profound.	Dorso-cranial border of axis neural spine beneath preceding muscle	Supraoccipital
Spinalis Capitis	Neural spines of posterior cervicals & anterior thoracics	Parietal
Longiss. Cap. (acting together)	Prezygapophyses of 4th cervical caudally	Squamosal, parietal (<i>Sphenodon</i>) Parietal (<i>Iguana</i>)
Sp. & Semisp. Cerv. (fused in <i>Iguana</i>)	Continuation of sp. & semisp. dorsi (<i>Iguana</i>) Longissimus & prezygapophyses of caudal cervicals (<i>Sphenodon</i>)	Sp. process of axis & postzygapophyses of atlas (<i>Iguana</i>) Proatlas and postzygapophyses of C 1 & 2 (<i>Sphenodon</i>)

Flexion of Head and Neck

MUSCLE	ORIGIN	INSERTION
Longus colli	Ventro-lateral surface of first 12 vertebrae; cervical & anterior thoracic ribs	Ventral surface of cervical vertebrae
Rect. Cap. Ant. (<i>Iguana</i>)	Ventral surface of 4th-7th cervical vertebrae and 7th cervical rib	Ventral surface of basioccipital
Longiss. Cap. (pars Trans. Cerv.)	Prezygapophyses of 4th cervical caudally	Spheno-occipital tubercle (<i>Sphenodon</i>)

Rotation and Abduction of Head and Neck

MUSCLE	ORIGIN	INSERTION
Obliq. Cap. Mag.	Lateral surface of the spinous process of the axis & dorsal surface of atlas neural arch	Opisthotic, prootic (<i>Sphenodon</i>) Opisthotic (<i>Iguana</i>)
Obliq. Cap. Infer.	Dorsal surface of the neural arch of the axis	Atlas postzygapophyses
Longiss. Cap.	Prezygapophyses of 4th cervical caudally	Squamosal, parietal (<i>Sphenodon</i>) Parietal (<i>Iguana</i>)
Longiss. Cap. (pars Trans. Cap.)	Prezygapophyses of 4th cervical caudally	Opisthotic, trans. proc. of ant. cervicals
Il-cost. Cerv. et Cap. (not separated in <i>Sphenodon</i>)	Longiss., lumbo-dorso fascia (<i>Iguana</i>)	Cervical ribs, atlas, basioccip. (<i>Iguana</i>)

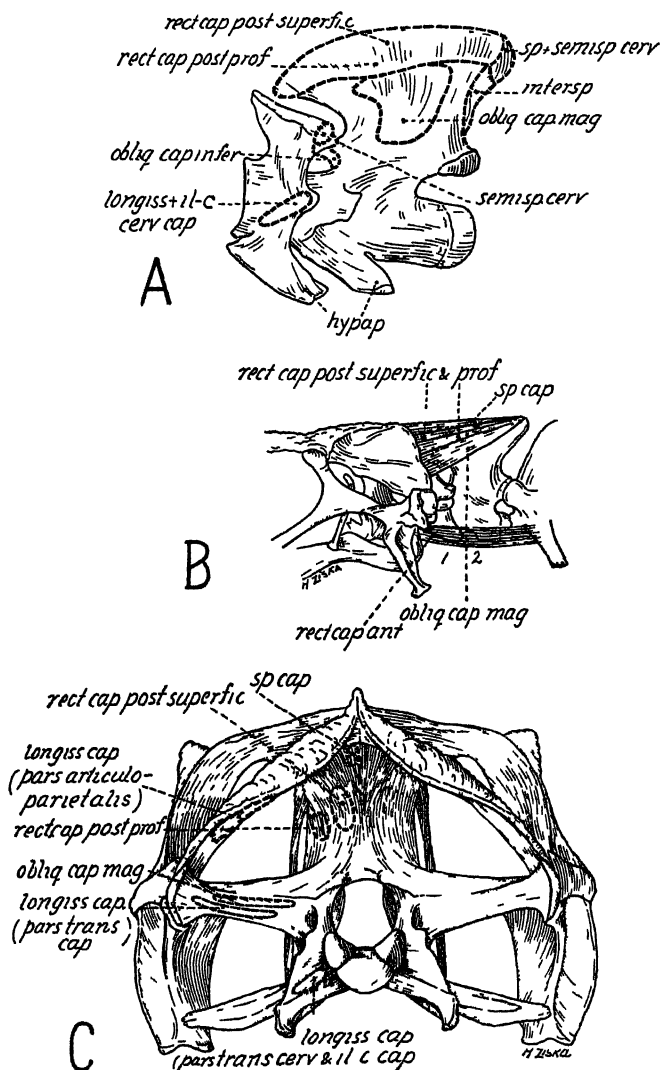


FIGURE 8. *Iguana*. A. Atlas-axis complex, showing origins of occipital muscles. B. Suboccipital and subvertebral muscles. C. Occipital view of skull, showing insertions of occipital and cervical muscles (semi-diagrammatic).

present and articulate with correspondingly small prezygapophyses on the axis.

The articular surface for the axis is concave and shows the same three articular areas as the facet for the condyle. These three areas

articulate with corresponding regions of the odontoid process which, as in all amniotes, is the atlas centrum fused with the axis. There is no proatlas. Transverse processes and ribs are absent.

The occipito-vertebral joint allows good rotation and lateral movements of the head but flexion and extension are limited. The freedom of movement at this joint is greater than in *Sphenodon*, probably due largely to the absence of a proatlas. The range of flexion is much greater than that of extension, which is soon stopped by contact of the occiput with the neural arch of the atlas. The range of all the movements is limited by ligaments and the action of antagonistic muscles.

The axis (FIGURE 8, A) is very similar to that of *Sphenodon* except that the neural spine is more expanded, indicating larger occipital muscles. The odontoid process has the same kind of transverse groove but the median dorsal point is more pronounced. There is a well developed hypapophysis and small but distinct prezygapophyses. The normal postzygapophyses face, as in *Sphenodon*, at about a 35° angle above the horizontal.

The atlanto-axial joint allows a combination of lateral movement and rotation about an oblique axis but flexion and extension are very slight. The articulation between the axis and the third cervical vertebra also permits these movements but allows more flexion and extension. The prococlous intervertebral joints facilitate these movements. Transverse processes and ribs are absent.

As would be expected from the similarity of the occiput and atlas-axis complex to those of *Sphenodon*, the occipital muscles (FIGURE 8, B) are essentially the same in morphology and function (TABLE 2) as those of *Sphenodon*.

The action of these dorsal extensor muscles is, as in Amphibia, opposed by that of ventral flexor muscles. The latter, however, are more highly differentiated than in the Amphibia and, instead of consisting of a single subvertebral muscle mass, are differentiated into a longus colli and a rectus capitis anterior. In possessing a rectus capitis anterior *Iguana* is more highly specialized than *Sphenodon*. The longus colli arises from the ventral side of the fourth through seventh cervical vertebrae and from the last three cervical ribs, and is inserted in the same place on the first three cervical vertebrae. The rectus capitis anterior arises from the centra of the fourth through seventh cervical vertebrae and from the seventh cervical rib and is inserted on the under surface of the basioccipital (FIGURE 15, C).

These muscles flex the head and neck and act as antagonists of the epaxial occipital and cervical muscles.

DIADECTES

This brief survey of the atlas-axis in these modern reptiles has laid the basis for an analysis of the complex in fossil forms, beginning with the Cotylosauria, the most primitive order of reptiles.

One of the best known cotylosaurs is *Diadectes* (FIGURE 9), a Permian form of which the Museum has considerable material. The skull is imperforate and in occipital view (FIGURE 9, C) is quite similar to the embolomere condition. There is a single large, somewhat heart-shaped condyle, which has a concave articular surface deeply pitted for the notochord. The sutures on the occiput are obliterated so that the boundaries of the individual bones can not be distinguished. However, comparison with Watson's (1918: 270) restoration of the occiput of the cotylosaur *Seymouria*, the most primitive known reptile, indicates that in all probability the condyle was tripartite and consisted of a large ventro-median basioccipital and two smaller dorso-lateral exoccipitals. The basioccipital part of the condyle slopes downward and forward while the more vertical exoccipital parts face posteriorly.

The atlas-axis complex of *Diadectes* (FIGURE 9, A) has been described and sketched for me in a personal communication from Dr. Olson, who has also figured it (1936: 280, fig. 8 K) previously. According to him, the right arch and spine of the atlas are entirely separate from those of the left side and in his sketch they appear to be well developed and posteriorly inclined. In side view they are slightly semicircular. The postzygapophyses are well developed and, when the arch is articulated with its central element, are nearly horizontal in position. The central element, which Olson does not identify with certainty, is large. According to Case (1911: 75), this element should be the first intercentrum and must necessarily be large because of the strong forward inclination of the basioccipital part of the condyle. Otherwise, the head would be carried at a sharp angle to the vertebral column. This central element is crescentic in shape and probably has on its cranial surface an articular facet for the basioccipital part of the condyle. According to Olson it has a facet for the capitulum of the first rib. In his sketch the lower part of the cranial surface of the atlas arch has what appears to be an articular facet. If so, this probably articulated with the flattened exoccipital part of the condyle.

Olson states that the proatlas is well developed and arises from a facet on the cranial surface of the atlas spinous process just above its base. Near the top it has a lateral facet which probably articulated with the occiput, although he has been unable to determine its exact position.

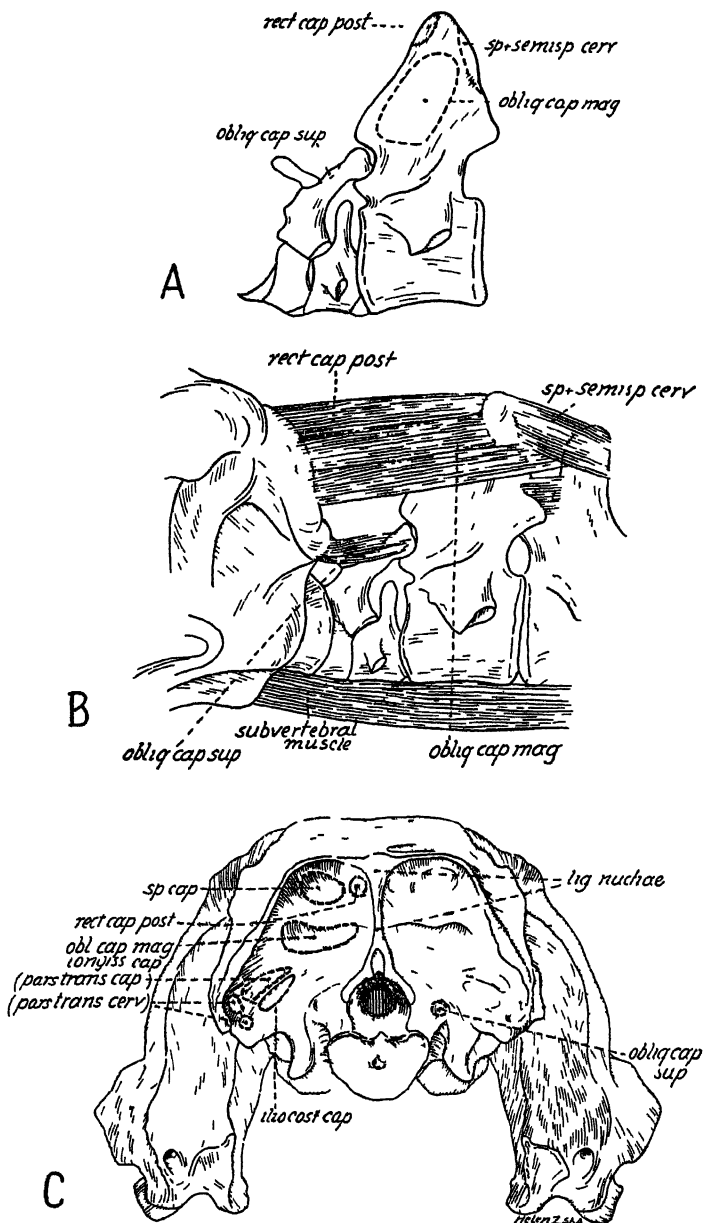


FIGURE 9 *Diadectes*. A Atlas-axis, showing origins of occipital muscles. Bones restored from personal description, sketch, and photograph by Dr. E. C. Olson. B. Restoration of suboccipital and subvertebral muscles. Modified from Olson. C Occipital view of skull, showing insertions of occipital and cervical muscles (semi-diagrammatic).

Olson has described, behind the first central element, a large bone with an anterior process simulating an odontoid, but he is not certain of its identity. Identification of this element as an odontoid would be in accord with the condition in *Seymouria*, as described and figured by Watson (1918: 277). An unusual condition, according to Olson, is the apparent possession of a neural spine by the second intercentrum.

The spinous process of the axis (FIGURE 9, A) is laterally compressed, antero-posteriorly expanded, and in side view tapers towards a point. The prezygapophyses are smaller than the well developed postzygapophyses, which are, according to Case (1911: 75) horizontal in position. The transverse processes are large. Both atlas and axis have double-headed ribs. The axis of *Diadectes* is more advanced than that of *Eryops* in having a larger, more expanded neural spine, which is on the way to becoming hatchet-shaped as in the higher tetrapods. This higher spinous process is correlated with a higher skull than in *Eryops*.

The range of movement in this type of atlas-axis would be limited. The occipito-vertebral joint was quite stiff but would permit rotation and lateral bending, depending in degree on how firmly the proatlas articulated with the skull and the atlas. As seen above, the atlas had not become differentiated into a ring that rotates about its own centrum as in the higher amniotes.

The skull of *Diadectes* is so solid and heavy that a strong occipito-vertebral joint, in conjunction with ligaments and muscles, was needed to support it. The horizontal position of the zygapophysial articulation between the atlas and the axis allows only lateral bending at this joint. The same is true of the articulation between the axis and the third cervical. The postzygapophyses of the latter however, are, according to Case (1911: 76), more obliquely placed, which permits a combination of lateral movement and rotation about an oblique axis. In addition, a little flexion and extension would also have been possible, both of which were absent or at least very slight in the preceding joints.

The neck of *Diadectes* was short and most of the movement would have been limited to a lateral swimming motion of the head. However, a slight rotation, flexion and extension of the neck and, indirectly, of the head could have occurred at the articulation between the third and fourth vertebrae. Posterior to this point extension of the neck would have been practically impossible, due to the close contact of the spinous processes.

Flexion would also have been slight, since the small space between the centra indicates rather thin intervertebral discs; consequently,

the centra would soon make contact with each other after a small range of flexion.

In *Sphenodon* and *Iguana* the enlarged neural spine of the axis is associated with, and provides a place of attachment for, special occipital muscles for moving the head. Since the neural spine of the axis in *Diadectes* is also expanded, it is reasonable to assume that the condition of the occipital muscles was similar to that in modern forms, although, because of the general primitive character of *Diadectes*, not as highly specialized. That the occipital muscles were less well developed is indicated further by the fact that the spinous process of the axis has not yet attained the hatchet shape so characteristic of higher amniotes. The rather strong resemblance of the neural spine of the axis to that of *Eryops* suggests a similar condition in the occipital muscles (FIGURE 9, B).

The low neural spines and swollen neural arches of the vertebrae posterior to the axis suggest that the epaxial musculature, especially the median spinalis system, was laterally expanded but not very deep or highly specialized.

Olson (1936: 276, 287, 300), from a study of the bones and by comparison with recent forms, has been able to restore the occipital and cervical muscles (FIGURE 9, B) and infers that they resembled those of *Eryops* quite closely.

On the ventral side of the skull, on each side of the occipital condyle, is a rather prominent depression which was probably for the cranial insertion of the subvertebral muscles. This musculature, since *Diadectes* is a primitive unspecialized form, was presumably not differentiated into separate muscles as in modern forms, but consisted of a single muscle mass on each side, running ventral to the spinal column from the tail to the head. Judging from the ventro-lateral depressions in which the muscle slips were inserted on the centra, the musculature must have been very powerful, since it apparently squeezed up a ventro-median keel that separated the two lateral masses (FIGURE 15, B). This muscle flexed the neck and head.

DIMETRODON

The next stage in evolution is represented by *Dimetrodon*, a rather specialized Permian pelycosaur. The skull was advanced over the cotylosaur condition by the acquisition of a single temporal fenestra, which places *Dimetrodon*, along with the mammal-like reptiles, in the subclass Synapsida.

In occipital view (FIGURE 10, C) the skull is higher and narrower

than in *Diadectes* and shows a single oval condyle with a convex articular surface. This tripartite condyle was composed of a ventro-median basioccipital part and two dorso-lateral exoccipital parts, the three bones contributing equally to formation of the condyle. The skull is still unfenestrated posteriorly and the supraoccipital region is inclined forward as in *Diadectes*.

The most obvious advance in the atlas-axis complex (FIGURE 10, A) over that of *Diadectes* is the great increase in the height and size of the neural spine of the axis, a change correlated with an increase in height of the skull.

The atlas, as in all amniotes, retained its temnospondylous condition. The first (atlas) intercentrum is the largest in the vertebral column. It is crescentic in shape and bears on the dorso-caudal surface an articular facet, slightly concave from side to side, that articulates with the ventro-cranial surface of the odontoid process. The cranial surface is deeply concave for the occipital condyle and has a slight ventro-median shelf. Dorsally there are two flattened facets for the neural arches of the atlas. Each lower lateral surface shows well defined articular facets for the atlantar ribs, which were fairly well developed.

The neural arches of the atlas were not preserved in my specimen but, according to Case (1907: 107), they were semicircular flattened elements quite separate from the centrum. They articulated posteriorly with the neural spine of the axis and ventrally with the atlas centrum (odontoid). They were not united dorsally and, as figured by Williston (1925: 101, f. 79), had concave cranial surfaces which continued the curves of the cranial surface of the atlas intercentrum, the three bones together making a cup for the occipital condyle. A pair of small bent proatlas elements were present on the anterior (cranial) surface of the atlas neural arches and connected the latter with the occiput. These elements were proportionately smaller than in *Diadectes*.

The odontoid (atlas centrum) is a large, somewhat crescentic bone whose cranial surface is very similar to that of a modern alligator. Seen from the front, it represents a flattened figure 8 and is convex dorso-ventrally. In the center of the dorsal margin of this surface is a small spout-like process with a deep notochordal pit immediately beneath it. On each side of this is a flat articular surface for the atlas neural arches. The saddle-shaped ventral half of the '8' articulated with the atlas intercentrum, while the central part of the upper half made contact with the occipital condyle. On the ventro-lateral sur-

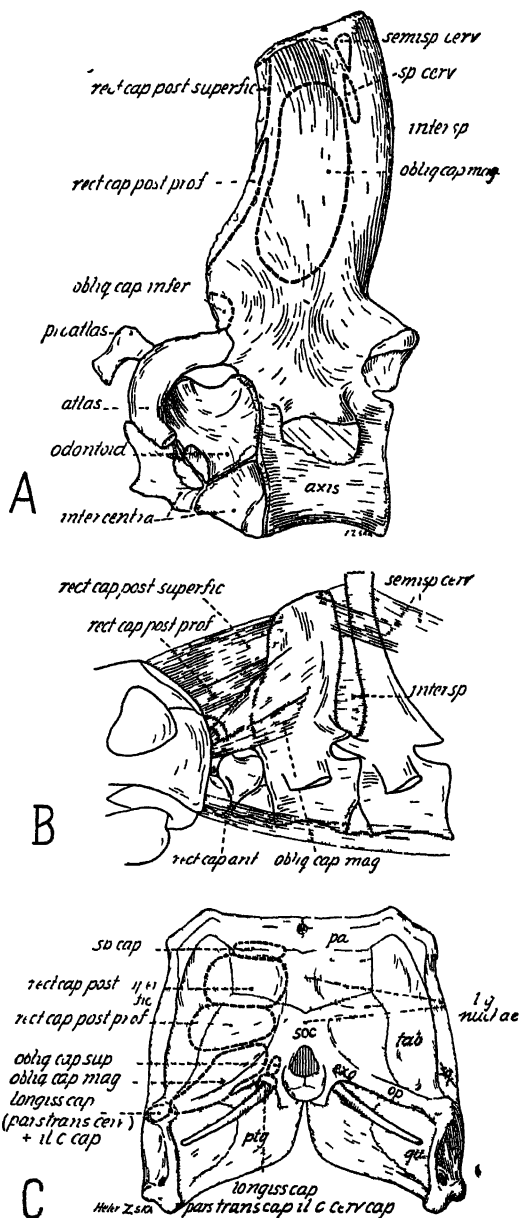


FIGURE 10 *Dimetrodon*. A. Atlas-axis, showing origins of occipital muscles (atlas neural arch and proatlas restored) B Restoration of suboccipital and subvertebral muscles Modified from Olson C. Occipital view of skull, showing insertions of occipital and cervical muscles Modified from Olson (semi-diagrammatic)

face is a facet for articulation with the tuberculum of the atlas rib. The caudal surface, which articulates with the axis, is slightly heart-shaped and has a concave face with a deep notochordal pit.

The atlas was more advanced than that of *Diadectes* in that its centrum had assumed the position and some of the functions of an odontoid process, although it had not yet fused with the axis and its neural arches and the proatlas are smaller.

The most notable advance in the axis of *Dimetrodon* (FIGURE 10, A) is the great increase in the size of the neural spine, although this is not yet hatchet-shaped. The postzygapophyses are large and set at about a 35° angle above the horizontal. The prezygapophyses, however, are very small and are practically horizontal in position, with flat articular faces.

The neural arch of the axis is fused with the centrum, which is elongated and curved downward so that the caudal face is lower than the cranial. The latter face seems to be divided into an upper surface for articulation with the odontoid and a lower one for the axis intercentrum. Due to the fact that my specimen was articulated I was unable to examine these surfaces completely but both are apparently concave and the upper one is probably pitted for the notochord. The caudal face of the centrum is oval, concave, and has a large, median notochordal pit. The transverse processes are well developed and articulate with the axis ribs.

The second (axis) intercentrum is crescentic and slightly smaller than the first (atlas) one. Cranially it articulates with the odontoid above and the atlas intercentrum below, while caudally it is in contact with the axis centrum. The lower side, near the caudal end, bears a facet for the capitulum of the axis rib.

The above described occipito-vertebral joint is nearer the type of modern reptiles than is that of the cotylosaur. Consequently, the motions permitted at this joint were more varied and freer than in *Diadectes*. The proatlas, being smaller, was probably not such a limiting factor as in the other fossil forms.

Virchow (1914: 107) has shown the types of movement possible in this region in a modern alligator. Since the proatlas, atlas, and odontoid of *Dimetrodon* resemble those of the alligator rather closely, they probably permitted similar types of movements at this joint. Rotation, flexion, extension, and some lateral movement were all possible at this joint but they varied in their range. Rotation occurred mostly at the occipito-atlantar joint, since the atlas is not differentiated into a ring rotating freely about the odontoid. However, some rotation

of the atlas about the odontoid was possible although it would have been slight and probably did not occur except, as in the alligator, during violent turning of the head.

Flexion and extension depended on the elasticity of the occipito-vertebral membranes and the articulation of the proatlas with the skull. Flexion, as in the preceding forms, had a greater range than extension, which was limited by the blocking action of the proatlas. Extension was stopped when the raising of the head had pushed the proatlas back until it abutted against the atlas arch. Consequently the larger the proatlas, the sooner its checking action takes effect.

Some lateral motion was also possible at the occipito-vertebral joint but most motion of this type probably involved turning the entire neck.

The flat, practically horizontal prezygapophyses of the axis would allow lateral motion but not much else at the atlanto-axial joint. Flexion and extension, if any, would be very slight, because the atlas was pushed or pulled backward or forward by the raising or lowering of the head.

The well developed, obliquely placed postzygapophyses of the axis would permit a combination of lateral movement and rotation about an oblique axis at the joint between the axis and the third vertebra. Also, there would be a greater range of flexion and extension between the axis and the third vertebra than between the axis and the atlas. The range of extension was limited by the close proximity of the spinous processes of the axis and the third vertebra, while flexion was limited by the joint capsules and the supraspinatus and interspinatus ligaments and dorsal extensor muscles.

The increased size of the neural spine of the axis probably means that the occipital muscles arising from it were larger and better developed than in *Diadectes*. Also, the closer resemblance of the entire atlas-axis complex to that of modern reptiles indicates that in all probability the occipital musculature was more highly differentiated and nearer to the modern condition than in the cotylosaurs.

On the antero-cranial border of the neural spine of the axis, separated by a slight ridge, are two depressed areas of muscle insertion which comparison with *Sphenodon* shows were probably for the rectus capitis posterior superficialis and profundus, respectively. These muscles (FIGURE 10, B) ran cranially and, according to Olson (1936: 301), were inserted beneath each other on the dermosupraoccipital, supraoccipital, and tabular. He also believes that the rectus capitis posterior profundus was probably in contact with the proatlas.

On the lateral surface of the spine of the axis is a large oval depression which, as in *Sphenodon* and *Iguana*, was probably for the obliquus capitis magnus. This was the largest of the occipital muscles and Olson has shown that it was inserted on the opisthotic.

On the cranio-ventral border of the axis spinous process is a prominent, semicircular depression which comparison with modern forms indicates was for the obliquus capitis inferior. According to Olson (1936: 302) this was a small muscle inserted on the postzygapophyses of the atlas. Presumably an obliquus capitis superior ran between the postzygapophyses of the atlas and the occiput but I was unable, due to the absence of the atlas neural arches in my specimen, to determine whether or not it was present.

The occipital muscles of *Dimetrodon* are more advanced than those of *Diadectes* in the separation of the rectus capitis posterior into a pars superficialis and a pars profundus.

The ligamentum nuchae was probably well developed, arising from the rugose tip of the neural spine of the axis and being inserted in the mid-line of the occiput. This ligament, as in all tetrapods possessing one, was the main support for the head; the muscles merely moved the head.

Dimetrodon is very specialized with respect to the neural spines and arches, which are very high and evidently supported a sail-like frill on the back. This feature implies that the medial epaxial muscles (spinalis-semispinalis system) were highly specialized and much better developed than in any of the preceding forms.

Olson (1936: 279, 289, 301) has restored the axial muscles of *Dimetrodon*. The functions of the occipital and cervical muscles were probably the same as those of the corresponding muscles in modern reptiles (TABLE 2).

As in preceding forms, the effect of the dorsal extensor muscles was opposed by the action of ventral flexor muscles. This subvertebral musculature, as indicated by the marked ventro-lateral constriction of the axial and other centra, must have been very powerful.

Since the atlas-axis complex, as well as the occipital musculature, approaches the modern condition more than in the cotylosaurs, it is reasonable to assume that the same held true for the subvertebral musculature. The latter, accordingly, would have been differentiated into a longus colli and a rectus capitis anterior. The longus colli probably arose, as in *Iguana*, from the ventral surface of the posterior cervicals and from the cervical ribs, and was inserted in similar fashion on the anterior cervical vertebrae. The rectus capitis anterior prob-

ably arose from the ventral surface of the centra of the posterior cervical vertebrae and was inserted on the underside of the occipital region of the skull. These muscles were the flexors of the head and neck

CYNODONTIA

The last reptile in the series is *Thrinaxodon*, of which the museum has a well preserved skull. This form is of particular interest as a member of the Cynodontia, the special group of reptiles that gave rise to the mammals.

The shape of the skull is very similar to that of the modern opossum, especially in occipital view (FIGURE 11, C). The low triangular occiput is imperforate, and shows an essentially mammalian condylar region. There are two well developed exoccipital condyles from which the basioccipital has been partly withdrawn, although it still articulates with the atlas intercentrum. The condyles are oval in shape and have convex articular surfaces.

The atlas-axis complex is absent in the museum specimen but it has been described and figured in *Galesaurus* (= *Thrinaxodon*) by Parrington (1934: 47). According to him, it (FIGURE 11, A) consisted of a proatlas, an atlas, and an axis as in *Dimetrodon*. The proatlas consisted of a pair of thin, rhomboidal plates overlapping the exoccipitals cranially and supported caudally by the articular processes of the atlas.

The atlas consisted of right and left halves, the upper median parts of which were broken in Parrington's specimen so that it is uncertain whether or not they met in the mid-line. Dorsally each half consisted of a plate which extended anteriorly under the outer half of the proatlas and rested posteriorly on the prezygapophyses of the axis. These plates, which were fastened to a stout wedge-shaped bone resting on the upper surface of the odontoid, showed well developed transverse processes projecting downward and backward.

According to Parrington, the first intercentrum was an oblong wedge-shaped bone with facets on its posterior corners. Apparently it was not large enough to articulate with the sides of the atlas, but lay against the odontoid and covered part of the basioccipital. An atlantar rib was present.

The spinous process of the axis was broken off in Parrington's specimen but it is well known in several other cynodonts and can be restored from them. A small projection went forward under the atlas and well developed postzygapophyses, set at about a 45° angle, were present. Below the postzygapophyses were small anapophyses that

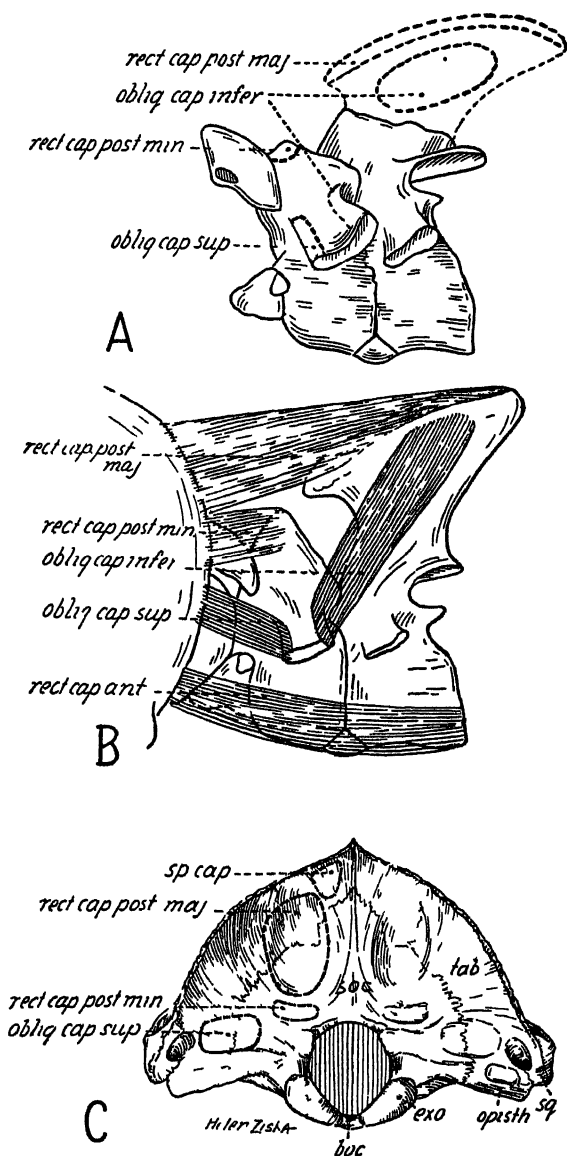


FIGURE 11 Cynodont A Atlas-axis of *Thrinaxodon* (*Galesaurus*) (from Parrington). Muscle origins restored B Restoration of suboccipital and subvertebral muscles C. Occipital view of skull, showing insertions of occipital and cervical muscles.

lay outside of and slightly above an anterior process arising from the base of the prezygapophyses of the third vertebra. The transverse processes were similar to those of the atlas. The centrum was constricted medially and apparently attached loosely to the odontoid except where the second intercentrum was wedged between them and partly fused with them.

Thrinaxodon is more advanced than *Dimetrodon* in being practically bicondylic and in having a hatchet-shaped spinous process on the axis, as is so characteristic of recent reptiles and mammals.

The occipito-vertebral joint of the cynodonts was functionally bicondylic and would permit flexion, extension, and lateral motions of the head on the vertebral column but practically no rotation. These movements depended on how tightly the proatlas articulated with the skull and the atlas. Flexion covered a greater range than extension, due to the blocking action of the proatlas, as in *Dimetrodon*. The cup-shaped condylar articulation would allow a small amount of lateral motion of the head but the separation of the condyles prevented rotation.

A cast of the cervical vertebrae of *Cynognathus* shows that the odontoid, especially its cranial articular surface, is very similar to that of *Tachyglossus*. Therefore it probably permitted the same types of movement as occur at this joint in modern monotremes. In accord with this line of reasoning the movements possible at this joint in cynodonts would have been chiefly abduction and a slight rotation of the head. The range of abduction, due to the unusual size of the odontoid articular facets for the atlas, would have been quite great. The practically horizontal position of the zygapophyses at this joint would also tend to limit movement here to abduction. Flexion and extension would have been practically out of the question because of the contact of the spinous process of the axis with the atlas arch and of the axis intercentrum, which was more or less wedged between and fused with the odontoid and the axis centrum. The oblique position of the zygapophyses between the axis and the third vertebra would have allowed flexion, extension, and a combination of lateral turning and rotation about an oblique axis to occur at this joint.

The above movements, as in the preceding forms, were produced by the action of the occipital and cervical muscles (FIGURE 11, B). These muscles, in keeping with the mammal-like character of the occiput, the atlas neural arch and transverse process, and the spinous process of the axis, were probably similar to those of a primitive mammal such as the opossum. The areas of muscle insertion on the occiput

are very faint but were distinguishable with the aid of a binocular dissecting microscope. The supposed origins of these muscles on the atlas and the axis are based on the conditions in the opossum.

The largest of the occipital muscles was the rectus capitis posterior major which probably arose as in the opossum, from the entire dorsal border of the spinous process of the axis. From here it ran forward to be inserted in a shallow oval depression on the dermo-supraoccipital, supraoccipital and tabular. Immediately beneath this was the rectus capitis posterior minor, which probably arose from the dorsal border of the atlas neural arch and was inserted in a small shallow oval depression on the supraoccipital.

The well developed transverse process on the atlas, an advance over *Dimetrodon*, is a mammal-like feature not found in recent reptiles. Its size suggests the presence of a well developed obliquus capitis superior and inferior and a rectus capitis lateralis, as in mammals, while the obliquus capitis magnus, the largest and most important occipital muscle in typical reptiles, was probably lost. The obliquus capitis superior presumably arose, as in the opossum, from the dorso-cranial border of the transverse process of the atlas and ran obliquely forward and inward to be inserted in a faintly marked depression on the exoccipital and opisthotic. The insertion indicates that this was the second largest occipital muscle inserted on the skull.

The obliquus capitis inferior probably arose, as in recent mammals, from the lateral surface of the spinous process of the axis and was inserted on the dorso-caudal border of the transverse process of the atlas. The rectus capitis lateralis probably ran forward from the transverse process of the atlas to be inserted on the occiput near the rectus capitis posterior minor, as it does in the opossum. The two recti muscles extended the head while the two oblique muscles abducted and rotated it.

A ligamentum nuchae probably ran between the spinous process of the axis and the mid-line of the occiput.

The postaxial cervical spines in *Cynognathus* are well developed but are not excessively long as in *Dimetrodon*. Their size implies that the cervical epaxial musculature was well developed. Since the cynodonts are nearer the mammals than the pelycosaur, the longissimus cervicis et capitis had probably lost its marked external segmentation, as Olson (1936: 278) says was the case for the spinalis-semispinalis in *Dimetrodon*. The ilio-costalis capitis had most likely been lost by now. I was unable to see any muscle scars on the cast of the cervical vertebrae of *Cynognathus* but the origins and insertions of the above muscles presumably were similar to their homologues in the opossum.

These muscles would extend and abduct the head and neck. Most of the rotational movement of the head may be attributed to the joint between the axis and the third cervical vertebra, which would permit this type of movement.

The subvertebral flexors of the head and neck must have been quite powerful in *Cynognathus*, in view of the ventro-lateral constriction of the centra. The centra also have a ventro-median keel which separated the musculature of the opposite sides.

This subvertebral musculature (FIGURE 15, D), since the cynodonts are so mammal-like in other respects, was probably differentiated into a rectus capitis anterior and a longus colli. It may be assumed that the rectus capitis anterior arose from the posterior cervical centra and was inserted on the ventral surface of the occipital region of the skull, while the longus colli originated from the posterior cervical ribs and the posterior cervical centra and ran forward to be inserted on the anterior cervical centra.

The cast of the spinal column of *Cynognathus* shows seven cervical vertebrae, probably the usual number in the cynodonts as it is in the great majority of mammals. These vertebrae have well developed neural spines and transverse processes which, according to Seeley (1895: 99), are for articulation with the tuberculum of a cervical rib whose capitulum articulated with the intercentral region. The neural arches are fused with the centra and, in *Cynognathus* at least, intercentra are present in the neck region. Both pairs of zygapophyses are well developed and are set at an oblique angle above the horizontal.

The above articulations would allow flexion, extension, and a combination of lateral movement and rotation about an oblique axis in the neck. Extension would have been limited largely by the anterior longitudinal ligament, which was presumably present as in recent tetrapods. The intercentra and posterior longitudinal and interspinus ligaments would have restricted flexion. Abduction and rotation were probably limited chiefly by the articular capsules.

MORPHOLOGY AND FUNCTION OF THE ATLAS-AXIS COMPLEX IN MAMMALS

With the rise of the mammals the tendencies, previously noted in reptiles, for the atlas to become a ring and for its centrum to fuse to the axis as the odontoid, are fully realized. The opposite halves of the neural arch are now fused with each other and with the atlas intercentrum to form a ring rotating about the odontoid. The latter has

become a peg-like pivot for the atlas and is completely fused to the axis. The proatlas has been lost, except in one or two cases, and the atlas is characterized by large, expanded transverse processes. The reptilian tendency toward the development of a large, hatchet-shaped spinous process on the axis culminates in the mammals. In these respects the atlas-axis complex is more specialized than in the lower tetrapods. These changes are correlated with changes in the occipital muscles.

The above, except in highly specialized aquatic forms like the Cetacea, is the usual mammalian condition of the atlas-axis complex in both recent and fossil forms. Therefore a discussion of its morphology and function may be confined to recent forms. For this purpose I have selected the opossum (*Didelphis*) as a primitive marsupial, the cat (*Felis*) as a typical placental, and man (*Homo*).

DIDELPHIS

The skull of the opossum resembles that of some cynodonts and the occiput (FIGURE 12, C) especially is very similar to that of *Thrinaxodon*. It differs from the latter, however, in having well developed paroccipital processes and in the complete withdrawal of the basioccipital from the condyles. The condyles are widely separated, oval convex eminences formed entirely from the exoccipital bones.

The atlas (FIGURE 12, A) is a complete ring, the neural arches and intercentrum being fused. The cranial surface is deeply concave and has two crescentic facets corresponding in shape to the occipital condyles with which they articulate. The caudal surface has two slightly concave facets for articulation with corresponding facets on either side of the odontoid. Inside the ventral arch of the atlas is a groove for the reception of the odontoid. The zygapophyses have been lost.

The atlas ring is quite wide cranio-caudally, but there is no neural spine. The transverse processes, however, are extremely well developed as flat, horizontally expanded blades that protrude laterally far beyond those of the other cervicals.

The axis (FIGURE 12, A) is characterized by a large expanded neural spine and by the odontoid process. The former is quite thick and is very similar in shape to that of the cynodonts. Anteriorly it overhangs the atlas arch, over which it glides during movements of the head. The peg-like odontoid has on each side an oval convex facet for articulation with the posterior surface of the atlas.

The prezygapophyses in my specimens are very small and do not articulate with the atlas. The postzygapophyses are well developed

and set at about a 33° angle above the horizontal. The articular faces are practically flat. The transverse processes are small and extend caudally.

The centra, in mammals generally, have practically flat articular

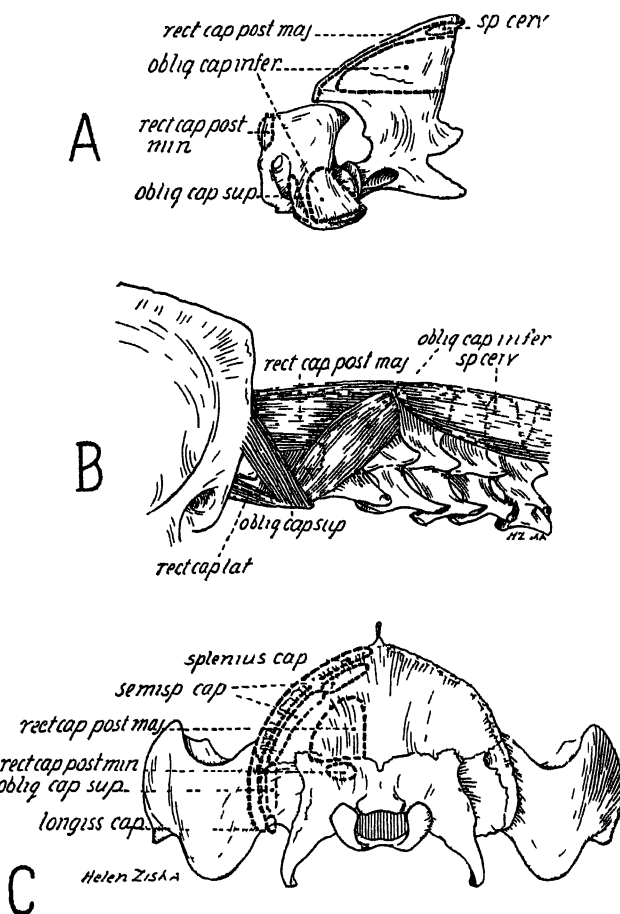


FIGURE 12. *Didelphis*. A. Atlas-axis, showing origins of occipital muscles B Occipital and subvertebral muscles C. Occipital view of skull, showing insertions of occipital and cervical muscles (semi-diagrammatic).

faces, although the cervical vertebrae in some of the long-necked ungulates show a ball-and-socket type of intervertebral joint.

The atlas-axis complex of mammals, because it is more specialized morphologically than that of the lower tetrapods, allows greater mo-

bility of the head. The occipito-vertebral joint of the opossum allows flexion, extension, and a small amount of abduction. Flexion has a greater range than extension, which can not go beyond the line of the vertebral axis due to the contact of the occiput with the atlas arch. The separation of the occipital condyles and the deeply concave form of the atlas facets prevent rotation and allow only a slight amount of abduction. Rotation, however, is very free at the atlanto-axial joint, where the ring-like atlas rotates about the odontoid. Flexion, extension, and abduction are very slight at this joint, owing to the shape of the articular facets, whose surfaces are reciprocally concavo-convex to a slight degree. Extension and abduction are further prevented by contact of the neural spine of the axis with the dorsal arch of the atlas.

The opossum has seven cervical vertebrae, the usual mammalian number. These, as in all mammals except the camels, are distinguished from other vertebrae by the possession of a foramen transversarium which transmits the vertebral artery and vein and a nerve plexus. The second through fifth cervical vertebrae have large, expanded, cancellous neural spines closely appressed to each other. The same is true for the neural arches of the fifth through seventh vertebrae but not for their spinous processes.

The result of the above condition is that practically all motion is eliminated in the region between the axis and the last cervical. This region acts as a single block and motions of the head and neck are restricted to each end of it.

Flexion and extension occur only at the occipito-vertebral joint, while rotation is confined to the atlanto-axial joint. However, the zygapophyses between the seventh cervical and the first thoracic vertebra are more oblique in position than in the preceding vertebrae. Consequently motion is freer at this joint, which allows flexion, extension, and a combination of abduction and rotation about an oblique axis.

The development of a more complex atlas-axis, allowing greater mobility of the head, is correlated with increased complexity of the occipital muscles (FIGURE 12, B). With the disappearance of the obliquus capitis magnus the obliquus capitis superior and inferior have increased in size and become the chief muscles for turning the head. These muscles are attached to the transverse processes of the atlas, the great expansion of which greatly increases their leverage.

The occipital and cervical muscles and their functions, which are practically the same in all mammals, are summarized in the following table.

TABLE 3

OCCIPITAL AND CERVICAL MUSCLES IN MAMMALS

Extension of Head and Neck

MUSCLE	ORIGIN	INSERTION
Splenius capitis	Ligamentum nuchae from C III posteriorly	Occipital crest (opossum, cat); mastoid proc. & below lat. $\frac{1}{2}$ sup. nuchal line (cat, man)
Splenius cervicis (both acting; absent in opossum & cat)	Supraspinus lig. & neural sp. of ant. thoracic vertebrae	Trans. proc. C I-CIII (man)
Spinalis cervicis	II-VII C spines	Sp. proc. of II & III cervicals
Longissimus cervicis	Trans. proc. of first 7 thoracic vertebrae	Trans. proc. II-V cervicals
Ilio-costalis cervicis (both acting)	II-V ribs	Trans. proc. last two cervicals
Semispinalis capitis	Trans. proc. first 8 thoracics & poszygapop. of II-VII cervicals	Med. and lat. part of occipital crest
Semispinalis cervicis	Articular proc. last 5 cervicals (cat); trans. proc. of first 5 or 6 thoracics	Sp. proc. of II-VII cervicals (cat); sp. proc. of II-V cervicals (man)
Rectus capitis posterior major	Sp. proc. of axis	Supraoccip. region of skull (opossum); below med. part of lambdoidal crest (cat); lat. part infer. nuchal line & below (man)
Rectus capitis posterior minor	Dorsal arch of atlas	Occiput below preceding (opossum, cat); med. part infer. nuchal line (man)

Flexion of Head and Neck

MUSCLE	ORIGIN	INSERTION
Longus colli	Centra of first 4 thoracics & trans. proc. of all cervicals except C I (cat); trans. proc. C III-V (man)	Ant. arch. of C I. trans. proc. V-VI C, centra II-IV cervicals (man)
Rectus capitis anterior major (Longus capitis)	Trans. proc. all cervicals; trans. proc. III-VI cervicals (man)	Basioccipital
Rectus capitis anterior minor	Ant. (ventral) border of atlas ring	Basioccipital

Rotation and Abduction of Head and Neck

MUSCLE	ORIGIN	INSERTION
Splenius capitis (acting singly)	Lig. nuchae from C III posteriorly & also from sp. proc. of CVII and first 3 or 4 thoracics (man)	Occip. crest (opossum); lambdoidal ridge (cat); mastoid proc. & below lat. $\frac{1}{3}$ sup. nuchal line (man)
Longissimus capitis	Trans. proc. first 5 thoracics, all C's except atlas	Mastoid process
Longissimus cervicis	Thoracic neural spines (cat); trans. proc. of upper 4 or 5 thoracics (man)	Trans. proc. of cervicals (cat); trans. proc. C II-VI (man)
Ilio-costalis cervicis (acting singly)	II-V ribs; II-VI ribs (man)	Trans. proc. last 2 cervicals; trans. proc. IV-VI cervicals (man)
Rectus capitis lateralis	Trans. proc. of atlas	Occiput below r. c. p. minor (opossum); fossa lat. to condyle (cat); jugular proc. (man)
Obliquus capitis inferior	Spinous proc. of axis	Trans. proc. of atlas
Obliquus capitis superior	Trans. proc. of atlas	Mastoid proc. & vent to lambdoidal crest (cat); occiput between sup. & infer. nuchal lines (man)

The ranges of the various movements of the head and neck are limited not only by the nature of the articulations, contact of the bones, and the action of antagonistic muscles but also by the spinal ligaments and those connecting the atlas and axis with each other and with the skull. These ligaments are fundamentally the same in all mammals and their attachments and the type of movements they check are presented in the following table.

TABLE 4
OCCIPITAL AND CERVICAL LIGAMENTS IN MAMMALS
Ligaments Limiting Extension

LIGAMENT	ATTACHMENTS
Anterior atlanto-occipital	Anterior atlas arch to anterior border of foramen magnum
Lateral (anterior oblique)	Trans. proc. of atlas to occiput lateral to condyles
Apical dental	Summit of odontoid to occipital close to foramen magnum
Transverse lig. of the atlas	Crosses atlas ring and holds odontoid in place
Joint capsule	Surrounds the joints

Ligaments Limiting Flexion

LIGAMENT	ATTACHMENTS
Posterior longitudinal (= tectorial membrane)	Centre of axis and 3rd vertebra to basilar part of occipital
Transverse lig. of the atlas	Crosses atlas ring & holds odontoid in place
Ligamenta flava	Connects the laminae of the axis with those of the 3rd cervical
Ligamentum nuchae	Tips of spinous processes of cervical vertebrae to occiput
Joint capsule	Surrounds the joints
Interspinous	Between the spinous processes.

Ligaments Limiting Rotation and Abduction

LIGAMENT	ATTACHMENTS
Alar ligaments	Apex of odontoid to occipital condyles
Anterior atlanto-axial	Ventral arch of atlas to front of axis centrum
Posterior atlanto-axial	Dorsal arch of atlas to arch of axis
Ligamenta flava	Connects laminae of the axis with those of 3rd cervical
Joint capsule	Surrounds the joints

FELIS

In the cat, a typical placental mammal, the brain case, containing a larger and more highly developed brain, is much more expanded than in the opossum. The occiput (FIGURE 13, C) is higher and the long paroccipital processes are absent. The occipital condyles are essentially the same as those of the opossum.

The atlas (FIGURE 13, A) is a ring but the ventral arch is smaller and narrower than in the opossum. This results in wide separation of the facets for the occipital condyles. These facets are deeply concave and extend a considerable distance in front of the ventral arch. The inner side of the ventral arch has a facet for the odontoid. On the caudal surface of the atlas are facets for the axis. These are smaller than the cranial facets and are oval and face inward. Their articular surface is slightly convex. There is no neural spine and the transverse processes are greatly expanded laterally.

The axis (FIGURE 13, A) is similar to that of the opossum except that the neural spine does not slope forward as much and does not touch the atlas arch. The facets for the atlas are large and crescentic, with convex articular surfaces. These, however, are not in complete accord with those of the atlas and therefore permit more varied movements at this joint. There are no prezygapophyses but the posterior

ones are well developed and arc at about a 43° angle above the horizontal. The transverse processes are greatly reduced and there are no ribs.

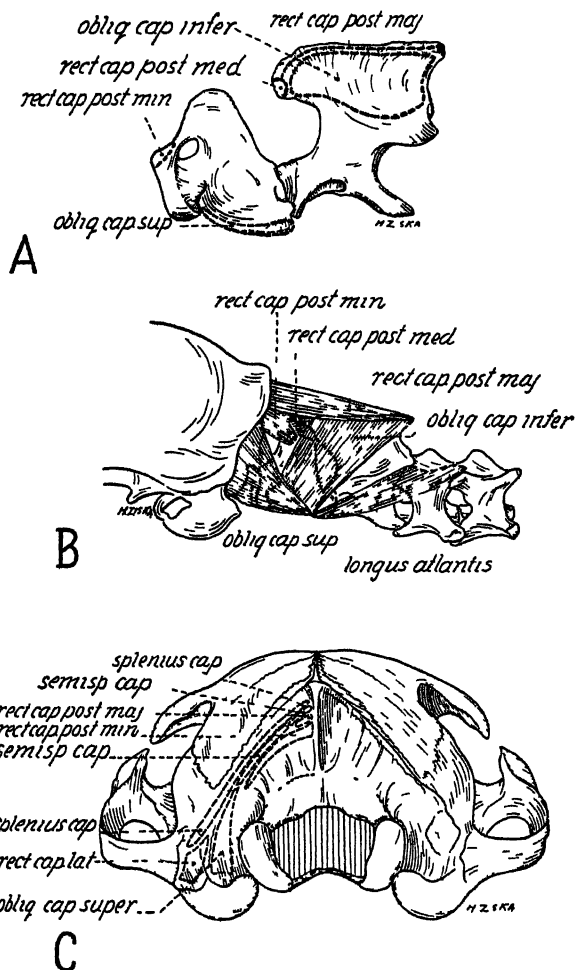


FIGURE 13. *Felis*. A. Atlas-axis, showing origins of occipital muscles B Occipital and subvertebral muscles C Occipital view of skull, showing insertions of occipital and cervical muscles (modified from Jayne)

The occipito-vertebral joint in the cat, as in the opossum, permits flexion, extension, and lateral movements. Extension can not go beyond a straight line, due to the contact of the occiput with the atlas

TABLE 5

	DORSAL CERVICAL AND OCCIPITAL					
	CRYPTO-BRANCHUS	RANA	RHACHITOMI ERYOPS	EMBOLOMERI	STEREO- SPONDYLI	SPHENODON
SPLENIUS <i>Splenius cervicis</i>						
<i>Splenius capitis</i>						
SPINALIS SYSTEM <i>Spinalis cervicis</i>			<i>Fused with semispinalis</i>			<i>Fused with semispinalis</i>
<i>Spinalis capitis</i>			<i>Present</i>			<i>Present</i>
<i>Semispinalis cervicis</i>			<i>Present</i>			<i>Present</i>
<i>Semispinalis capitis</i>						
LONGISSIMUS SYSTEM <i>Longissimus cervicis et capitis</i>	<i>Longissimus capitis (extension of longissimus dorsi)</i>		<i>3 Parts:-- Articulo-parietalis Transversalis capitis & cervicis Longissimus cervicis</i>	<i>Longissimus capitis (extension of longissimus dorsi)</i>	<i>Longissimus capitis (extension of longissimus dorsi)</i>	<i>3 Parts:-- Longissimus cervico-capitis Transversalis cervicis Small slip inserting on sphenio-occipital tubercle</i>
ILIO-COSTALIS SYSTEM <i>Ilio-costalis cervicis</i>			<i>Fused with transversalis capitis and cervicis</i>			<i>2 Parts:-- cervicis capitis</i>
SUBOCCIPITAL MUSCLES <i>Rectus capitis posterior</i>	<i>Present</i>		<i>Present</i>	<i>Present</i>		<i>2 Muscles superficialis profundus</i>
<i>Obliquus capitis</i>		<i>Superior</i>				<i>2 Muscles magnus inferior</i>
<i>Rectus capitis lateralis</i>		<i>Present</i>				

TABLE 5—Continued

MUSCLES IN TYPE FORMS						
IGUANA	DIADECTES	DIMETRODON	THRINAXODON	DIDELPHIS	FELIS	HOMO
						<i>Present</i>
				<i>Present</i>	<i>Present</i>	<i>Present</i>
<i>Fused with semispinalis</i>	<i>Fused with semispinalis</i>	<i>Present</i>		<i>Present</i>	<i>Present</i>	<i>Present</i>
<i>Present</i>	<i>Present</i>	<i>Present</i>	<i>Present</i>			
<i>Present</i>	<i>Present</i>	<i>Present</i>	<i>Present</i>	<i>Present</i>	<i>Present</i>	<i>Present</i>
			<i>Present</i>	<i>2 Parts:— complexus biventer</i>	<i>2 Parts:— complexus biventer</i>	<i>Present</i>
<i>4 Parts:— Articulo- parietalis Transversalis capitis Transversalis cervicis Longissimus cervicis</i>	<i>4 Parts:— Articulo- parietalis Transversalis capitis Transversalis cervicis Longissimus cervicis</i>	<i>4 Parts:— Articulo- parietalis Transversalis capitis Transversalis cervicis Longissimus cervicis</i>	<i>Present</i>	<i>Present</i>	<i>Present</i>	<i>Present</i>
<i>2 Parts:— cervicis capitis</i>	<i>2 Parts:— cervicis capitis</i>	<i>3 Parts:— cervicis capitis Small median part</i>	<i>Present</i>	<i>Present</i>	<i>Present</i>	<i>Present</i>
<i>superficialis profundus</i>	<i>Present</i>	<i>Present (superficialis & profundus possibly separate)</i>	<i>2 Muscles major minor</i>	<i>2 Muscles major minor</i>	<i>2 Muscles major minor</i>	<i>2 Muscles major minor</i>
<i>2 Muscles magnus inferior</i>	<i>2(3) Muscles magnus superior (inferior possibly present)</i>	<i>3 Muscles magnus superior inferior</i>	<i>2 Muscles superior inferior</i>	<i>inferior</i>	<i>2 Muscles superior inferior</i>	<i>2 Muscles superior inferior</i>
			<i>Present</i>	<i>Present</i>	<i>Present</i>	<i>Present</i>

arch. Flexion has a much greater range and, as far as the bones themselves are concerned, the head could be flexed a trifle beyond a right angle to the axis of the spinal column before the atlas came in contact with the basioccipital. A slight amount of abduction is also possible at this joint, since one condyle glides down in the atlas cup while the other glides up.

The atlanto-axial joint permits rotation, flexion, extension, and abduction. Rotation is the freest and the most important. In this movement the posterior articular facet of the atlas, on the side towards which the face is turned, glides dorso-caudally while the opposite facet glides ventro-cranially on its axial facet. The convex facets of the axis also allow flexion, extension, and some abduction. Extension is limited by the contact of the spinous process of the axis with the dorsal arch of the atlas, while flexion and abduction are limited chiefly by ligaments.

All these movements are effected by the action of the occipital muscles (FIGURE 13, B) and are limited by ligaments (TABLE 4). The checking effect of the ligaments usually takes place before the contact of the bones.

The cat, like the opossum, has seven cervical vertebrae but the individual bones are quite different. In the cat the neural spines of the first few vertebrae behind the axis are reduced to a low ridge, and are only of medium height in the posterior cervicals. The transverse processes are also reduced and there are no ribs. The zygapophyses are set at about a 43° angle above the horizontal and have practically flat articular surfaces.

The total effect of all these differences is that the neck, in the region between the axis and the first thoracic vertebra, is more mobile than the corresponding region in the opossum. The oblique position of the zygapophyses permits flexion, extension, and abduction combined with a slight amount of oblique rotation at these joints. The small size of the neural spines permits extension, which is checked by the anterior longitudinal ligament or the eventual contact of the neural arches of the vertebrae. Flexion is restricted by the dorsal spinal and interspinal ligaments, while abduction and rotational movements are checked by contact of the bones and the joint capsules, which are larger and looser than in any other part of the spinal column.

The dorsal extensor muscles of the head and neck are opposed by the action of the ventral flexors, whose condition in the cat can be considered typical for mammals. These muscles are summarized in TABLE 3 (see FIGURE 15, E).

HOMO

In man upright posture has brought about many changes in the skull and cervical vertebrae. The facial part of the skull has been inclined downward so that it forms practically a right angle with the axis of the spinal column.

The skull is now balanced on the upper end of the spinal column so that the foramen magnum and the occiput are in a nearly horizontal plane and, consequently, face downward instead of posteriorly as in pronograde animals. The occiput (FIGURE 14, C) is oval in outline and the supraoccipital portion is greatly expanded. This provides greater area for the attachment of the occipital and cervical muscles. The occipital condyles are proportionately smaller in relation to the foramen magnum than in the cat and are elongated oval eminences well separated from each other.

The atlas (FIGURE 14, A) is a thin bony ring with no spinous process but with well defined, though short, transverse processes having a large foramen transversarium. The occipital facets of the atlas are well separated and deeply concave. Those for the axis are smaller, more circular in shape, and only slightly concave. In the center of the inner surface of the ventral atlas arch is a slightly concave facet for the odontoid. There are no zygapophyses.

The axis (FIGURE 14, A) is a short vertebra with a large odontoid. The spinous process, in contrast to the condition in pronograde mammals, is not a hatchet-shaped blade but is rather short and bifurcated at the tip. The transverse processes are short.

The facets for the atlas are broad ovals with practically flat articular surfaces. There are no prezygapophyses. The small postzygapophyses, more dorsally placed than the facets for the atlas, have nearly flat articular surfaces and are oblique in position like those of the posterior cervical vertebrae.

The occipito-vertebral joint in man permits extensive flexion and extension movements, but no rotation and only very slight abduction motions. Flexion has a greater range than extension, which would soon be stopped by contact of the occiput with the posterior (dorsal) arch of the atlas. Flexion would eventually be stopped by contact of the basioccipital with the anterior (ventral) arch of the atlas. Of course, as in the preceding forms, these movements would be checked by ligaments (see TABLE 4) before they had gone this far. The abduction motion consists of a slight lateral rocking movement of the head on the atlas. The muscles producing these movements are presented in TABLE 3 and FIGURE 14, B.

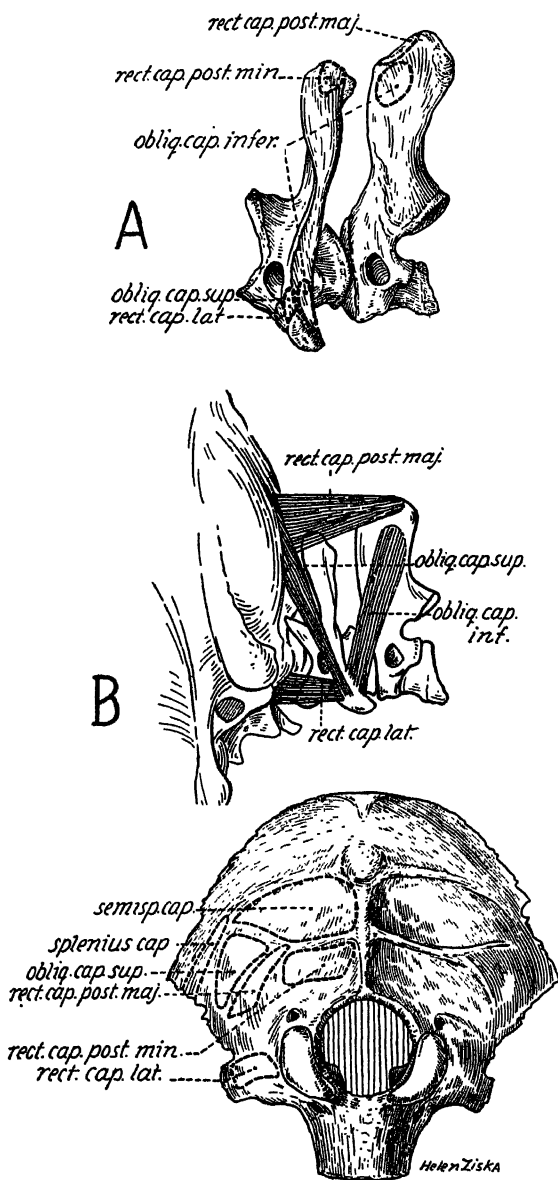


FIGURE 14. *Homo*. A. Atlas-axis, showing origins of occipital muscles. B. Occipital muscles (diagrammatic). C. Occipital view of skull, showing insertions of occipital and cervical muscles (modified from Gray).

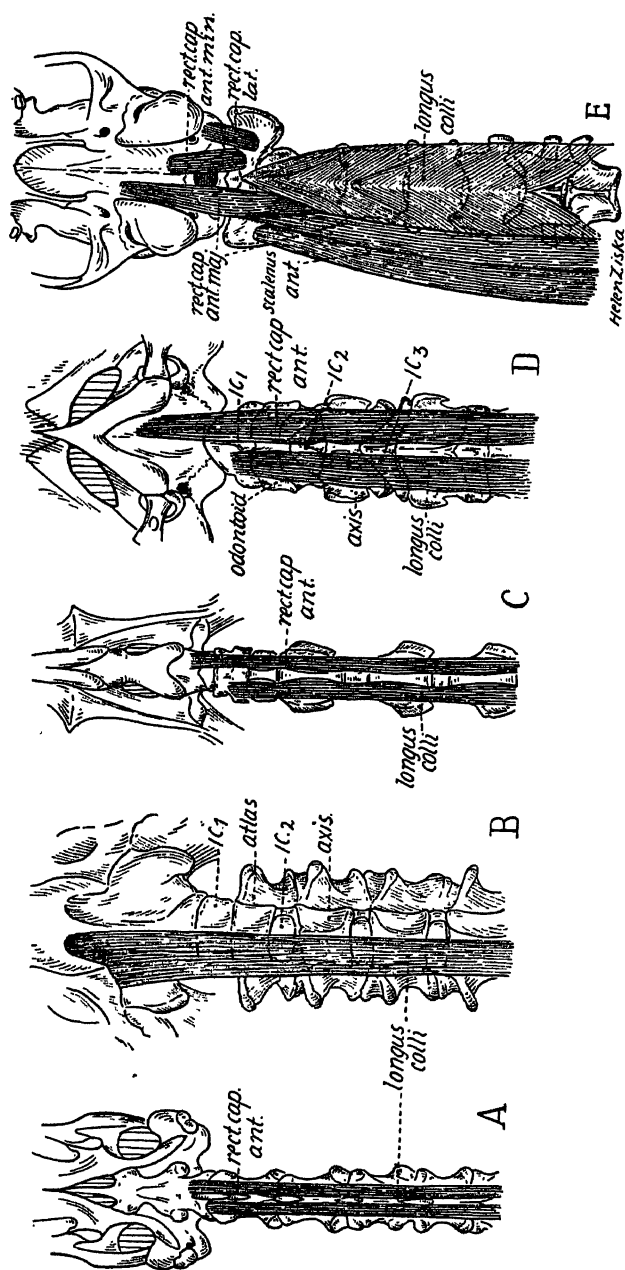


FIGURE 15. Subvertebral muscles of various types (semi-diagrammatic). A. *Sphenodon*. B. *Diadectes* (restored). C. *Iguana*. D. *Cynodont* (restored). E. *Felis*.

The chief motion at the atlanto-axial joint is rotation, although flexion, extension, and abduction are also present. The rotation, which occurs about a slightly oblique angle, is due to the antero-posterior movement of the atlas on the convex surface of the facets of the axis, which are directed supero-laterally. In this motion the head and atlas move together on the axis and odontoid. Flexion, extension, and lateral (abductional) movements are freer than in most other vertebral joints. The range of all these movements is restricted by ligaments (TABLE 4).

Man has seven cervical vertebrae, all of which except the seventh have short neural spines. These, except in the Australian native according to Jones (1938: 411), are bifid at the tip. The short transverse processes are bifurcated distally. The zygapophyses, set at about a 45° angle above the horizontal, have practically flat articular surfaces. The angle of these processes permits flexion, extension, and a combination of abduction and rotation.

Movements, except between the axis and the third cervical, are freer in the neck than in any other part of the spine. The restriction between the axis and the third cervical is due to the ventral lip of the caudal surface of the axis centrum, which limits flexion. Rotation in the neck, due to the oblique position of the zygapophyses, occurs about an oblique axis. According to Fick (1911: 85) the various types of movement in the neck have the following ranges:

Flexion.....	about 90°
Extension.....	about 90°
Lateral inclination.....	about 30°
Rotation.....	about 45°
Inclination and rotation.....	about 90°

These movements are restricted by the various ligaments listed in TABLE 4 and also, as in all tetrapods, by the compressibility of the intervertebral discs.

DISCUSSION

In the preceding sections the morphology and functional evolution of the atlas-axis complex have been traced in some detail from the unspecialized fish condition through the amphibians and reptiles to the specialized mammalian condition. Also, two types of skulls have been noted—the flat type characteristic of amphibians generally and the high type characteristic of reptiles and mammals.

The contrast between the atlas-axis complex of amphibians and that of amniotes seems to be related to these two types of skulls. A

flattened atlas-axis complex with a short but longitudinally elongated neural spine on the axis is usually associated with a flat skull. This type is seen in *Cryptobranchus*. An atlas-axis complex with a high, more or less hatchet-shaped spinous process on the axis, as seen in typical reptiles and mammals, is usually associated with a high, narrow skull. In other words, both the atlas-axis complex and the skull are correspondingly heightened or flattened by the same process.

The high skull of the reptiles and mammals is much nearer the primitive tetrapod type, as represented by the Carboniferous embolomeres, than is the flat skull of the later fossil and recent amphibians. In fact, as will be shown, the modern and later fossil amphibians are aquatic, secondarily specialized types.

The primitive embolomorous amphibians of the Carboniferous, e.g., *Palaeogyrinus*, represent the probable ancestral tetrapod condition or at least something approximating it. These forms had a relatively high skull similar to that of their crossopterygian contemporaries. This general type of skull has been retained by the great majority of reptiles and mammals.

The amphibians, however, have evolved in the other direction and show progressive flattening of the skull. This flattening began in the Rhachitomous amphibians of the Permian (e. g., *Eryops*) and culminated in the excessively flattened skulls of the Permian lepospondyles (e. g., *Diplocaulus*) and the Triassic stereospondyles (e. g., *Plagiosternum*). It is also characteristic of the modern Caudata and Salientia. The atlas-axis complex of these forms has also shared in this flattening process and shows transversely widening and reduction of the neural spine of the axis to a low median ridge.

A progressive modification of the condyle from the ancestral condition accompanies this change in the amphibian occiput. In the embolomeres there is a single tripartite condyle with a convex articular surface. This condyle is formed by the two exoccipital bones and the single basioccipital, which is the dominant element. In the later forms the basioccipital becomes progressively reduced and contributes less and less to the formation of the condyle. This process continues until finally, in the Permian and Triassic forms as well as in the modern ones, the basioccipital has completely disappeared, leaving two well separated exoccipital condyles paralleling the mammalian condition.

The reptiles, however, with the exception of the advanced cynodonts, have retained the single oval tripartite condyle up to the modern forms. The cynodonts became almost completely bicondylic functionally, although the basioccipital bone is retained. The mammals

have further developed the bicondylic condition initiated by the cynodonts and also have retained the basioccipital bone.

The bicondylic mammalian condition is a stronger joint than the monocondylic, ball-and-socket type of most reptiles, since the skull and spinal column are in contact at two points instead of one. According to Fick (1911: 113), this distribution of movements between the head and the spinal column over two joints, in contrast to the single joint in monocondylic types, increases the firmness of the occipito-vertebral union.

These movements—chiefly flexion, extension, and a very slight amount of lateral inclination and rotation in the bicondylic mammals—take place, because of the physical nature of the articulations, about two main axes, one sagittal and the other transverse. In the monocondylic, ball-and-socket, occipito-vertebral joint of typical reptiles the movements between the head and spinal column can take place about several axes.

Only mammals and amphibians, have a true bicondylic condition, although some of the higher cynodonts were functionally bicondylic. This condition, however, seems to be a case of convergence and to have arisen independently in the two groups.

In typical mammals, which have the body raised well off the ground and are more or less cursorial, a firmer occipito-vertebral joint is needed to help keep the head from bobbing around too much in running.

In the Caudata, most of which are aquatic or semi-aquatic, the firmer bicondylic type of occipito-vertebral joint seems hardly necessary but the Salientia need a firm occipito-vertebral joint to keep their heads from bobbing when they jump.

The skull of the modern amphibians is secondarily degenerate in including only ten cranial nerves, inasmuch as the *Embolomeri* and several other fossil forms (*Trimerorhachis*, *Eryops*, and *Erpetosaurus*) had, according to Watson (1919: 59), Broom (1913: 573, 584), and others, twelve cranial nerves. The presence of twelve cranial nerves in *Erpetosaurus* is particularly significant, in that it is one of the most primitive genera of Phyllospondyli, the order generally considered ancestral to the Salientia and Caudata. The loss by the modern amphibia of the hypoglossal as a cranial nerve may be, according to Gadow (1933: 67), correlated with the disappearance of the basioccipital, which was present in these forms. Thus the exoccipital portions of the originally tripartite single condyle remained as two separate condyles in modern amphibia.

The skull of the Salientia and the Caudata is also highly fenestrated in contrast to the imperforate skull of the embolomeres and the rhachitomes. This fenestration has been produced largely by the great reduction in number of skull elements, since the dermo-supraoccipital, supraoccipital, tabular, supratemporal, intertemporal, postfrontal, postorbital, prefrontal (retained in some species), interparietal, lacrymal (retained in some species), basioccipital, and basisphenoid have been lost.

The Caecilia differ from other modern Amphibia in having a solid compact skull and long curved ribs. Both of these features may be correlated with their burrowing habits. Also, in some genera they retain the ectopterygoid bone, a very primitive element that was early lost by the phyllospondyles.

A definitive neck first appeared in the Amphibia with the separation of the pectoral girdle from the skull and its later posterior migration. In some fish, *e. g.*, *Squalus*, the pectoral girdle is also separated from the skull but there is no special modification of the spine or muscles to differentiate this region as a neck. According to Watson's (1926: 236) restoration of *Eogyrinus*, an early Carboniferous embolomere, the pectoral girdle was still connected to the skull by a post-temporal bone as in fish.

In the later stegocephalians and early reptiles the pectoral girdle lies immediately behind the skull, and probably helped support the heavy head and prevented lateral movements of it. In the early amphibians separation of the girdle and the skull allowed lateral swinging of the head and neck, but this was prevented in the later stegocephalians by the development of separate exoccipital condyles. The same is true of the modern Amphibia.

The Amphibia have never developed a complex atlas-axis or a long flexible neck as have the reptiles and mammals. This is probably due to the fact that they are primarily an aquatic or semi-aquatic group. With the marked exception of the plesiosaurs, aquatic forms do not usually possess a highly mobile occipito-vertebral joint or a long, flexible neck, for such structures would be a disadvantage in swimming.

The atlas-axis complex of modern forms has lost the proatlas, which, as already noted, was probably present in the embolomeres and rhachitomes, and the entire complex has become simplified. Both vertebrae are flattened and the neural spine of the axis is reduced to a low ridge.

The dedifferentiation of the vertebral column in Caudata to a string of secondary polyisomeres and the extreme shortness of the

column in Salientia are both specialized conditions quite different from the primitive condition in the embolomeres and rhachitomes.

The short rod-like ribs of the modern Amphibia are also in marked contrast to the long curved ribs of embolomeres. In the latter forms the ribs gave the body an oval outline similar to that of their crosspterygian contemporaries and quite different from the flat depressed bodies of the modern frogs and salamanders.

The undifferentiated, metameric structure of the epaxial musculature in the Caudata is simply a functional adaptation associated with retention of the fish-like method of locomotion. The aquatic forms swim by lateral undulations of the laterally compressed tail, the legs being held against the sides of the body. In crawling on the bottom or on land they practically swim over the surface by lateral undulations of the trunk, produced by an alternate series of contractions of the axial muscles. In this type of motion independent fore and aft movement of the legs is not necessary in walking. In all probability, according to Watson (1926: 202), this is the type of locomotion characteristic of the primitive embolomeres.

In contrast to the simplicity of the epaxial musculature in the salamanders is the complexity of the hypaxial muscles. The latter, in some forms, are differentiated into superficial and deep external obliques, an internal oblique, a transversus, and a superficial and deep rectus. According to Noble (1931: 249), this complexity is related to their functions in supporting the viscera and in respiratory movements.

The Salientia are even more removed from the ancestral type. The lacrymal and prefrontal are never present and the spinal column has been reduced to nine free vertebrae, the smallest number in any vertebrate. Ribs are absent in the adult but Mookerjee (1930: 170, 175) has found them in the embryo in *Rana temporaria* and *Bufo melanostictus*. The well developed saltatorial hind legs of the frogs are also highly specialized and are quite different from the weak legs of the salamanders and embolomeres. According to Noble (1931: 251), the superficial epaxial muscles of the frogs have lost their primitive function of bending the spine laterally and have assumed the new one of holding up the head in jumping and of bending the vertebral column upward in the "warning" attitude. The epaxial muscles are also more differentiated than in the Caudata (FIGURES 3, 4).

All these differences from the primitive tetrapod condition, as represented by the embolomeres, indicate that the modern Amphibia are not primitive forms, as held by many anatomists and zoologists,

but are really specialized, secondary types. This view is apparently held by Noble (1931: 12), who says that in many respects modern Amphibia are more specialized than the reptiles. Dr. William K. Gregory tells me that he also has suspected this for some time.

The earliest reptiles were sprawling forms with the head and body close to the ground and the legs extending out laterally. With the rise of the higher theromorphs and the cynodonts several changes occurred. The better developed legs were brought in beneath the body to raise it and the neck and head well above the ground. This gave the animal a much wider horizon of vision and necessitated the development of a more mobile occipito-vertebral joint for turning the head on the spinal column, and a longer, more flexible neck for moving the head as a whole. The raising of the body and the head from the ground was followed by changes in position of the orbits in the skull. In aquatic forms the orbits are more dorsal in position but in truly terrestrial forms the orbits are more laterally placed, so that the eyes are in a better position for surveying the increased horizon.

In aquatic forms the support of a broad flat skull involves no great mechanical problems in view of the buoyant effect of the surrounding water. In entirely terrestrial forms, however, support of this type of skull for any length of time would be very difficult since the occiput is not high enough to provide much area of insertion or leverage for the ligaments and muscles supporting and moving the head. The modern crocodiles and Caudata, both semi-aquatic forms with broad flat skulls, never support the head off the ground for a long period of time.

In reptiles and mammals the occiput is nearer the primitive embolomere type and expansion has occurred dorsally instead of laterally as in the amphibians. Consequently there is a much larger area for the insertion of, and to provide leverage for, the occipital and cervical muscles. The vertical expansion of the occiput has been accompanied by a similar expansion of the neural spine of the axis. This increased height of the axis spine gives greater leverage to the occipital muscles arising from it. The efficiency of the occipital muscles was also increased in other ways by the dorsal expansion of the spinous process of the axis and of the occiput. The muscles were increased in length as they became inserted higher above the occipital condyles than formerly. This afforded a greater range of contraction, since the distance a muscle can contract is directly proportional to the length of its fibers.

The increase in insertion area probably indicates an increased number of fibers in the individual muscles with a consequent increase

in the force exerted since the latter depends upon the number of fibers or the cross-section area of all the fibers. These changes increased not only the force but also the amount of work the muscles were able to do, because the force is proportional to the volume of the muscle divided by the length of its fibers, while the amount of work a muscle can do is proportional to the volume.

The high occiput also increases the effectiveness of the long extensor muscles of the neck, since they are inserted nearer the mid-line so that the direction of the force of contraction is more in line with the axis of the spinal column.

The necessity for increased mobility of the head on the vertebral column had a more profound effect on the atlas than on the axis. These changes have already been presented in some detail in the preceding sections but will be briefly reviewed here: (1) the proatlas is reduced and finally lost; (2) the atlas spinous process is lost and the opposite halves of the neural arch fuse with each other and with the atlas intercentrum to form a ring; (3) with the rise of the reptiles the anterior facets on the atlas become markedly concave for the reception of the ball-like occipital condyle; (4) the atlas centrum becomes separated from its neural arches, moves posteriorly to a position between the atlas and the axis and finally fuses with the axis centrum to form the odontoid; and, (5) prominent lateral processes grow out from the sides of the atlas. These changes begin in the early reptiles but attain their complete development only in the mammals.

All reptiles, with the exception of the cynodonts, are monocondylic and nearly any type of movement can occur at their occipito-vertebral joint. Consequently the atlas does not become a completely closed ring nor the odontoid a perfect peg about which the former can rotate.

However, the development, in the cynodonts and their descendants the mammals, of the bicondylic condition necessitated more changes in the atlas, for the reason that rotation was no longer possible at the occipito-vertebral joint. To provide for this motion the atlas becomes completely ring-like and the odontoid a perfect peg for rotation of the atlas and the head. In mammals the atlas also develops large transverse processes for the obliquus capitis muscles. Atlantar transverse processes are absent in typical reptiles.

The loss of the spinous process of the atlas was necessary, since a large spine would not only prevent rotation by colliding with the neural spine of the axis, which overhangs the atlas arch, but would also limit extension of the head by contact with the occiput.

In reptiles the chief muscle for turning the head is the obliquus

capitis magnus, which arises from the axial spine and is inserted on the occiput. In mammals, however, this muscle has disappeared and its functions are taken over by the obliquus capitis superior and inferior. These muscles are fastened to the atlantar transverse processes, which protrude far beyond those of the other cervicals and provide great leverage for the muscles.

The splenius also appears, for the first time, in mammals. It may have arisen as a superficial epaxial cervical muscle split off from the underlying longissimus capitis. It is not present as such in reptiles but in *Sphenodon* the dorsal part of the longissimus capitis (pars articulo-parietalis, Nishi) is in general appearance and in the direction of its fibers very similar to the splenius of mammals.

The fate of the obliquus capitis magnus in reptiles and the origin of the obliquus capitis superior and inferior in mammals is uncertain. According to Nishi (1919: 242), *Varanus* has three obliquus capitis muscles, magnus, superior, and inferior. However, I was unable to distinguish them and apparently they are simply differentiations of the obliquus capitis magnus muscle mass. In *Iguana* only the obliquus capitis magnus appears to be present.

It has been suggested to me by Mr. Harry Raven that, from this condition with a single large obliquus capitis muscle mass, the obliquus capitis superior and inferior of mammals could have been derived in the following manner. There was first a shift in the attachment of some of the fibers of the general muscle mass to insertions on the transverse processes of the atlas, which at first were very small. The attachment of these fibers stimulated the transverse processes to hypertrophy, so that they pushed out into the obliquus muscle mass. This process continued until the obliquus mass was completely intersected and divided into two halves. The cranial half, running between the transverse process and the skull, became the obliquus capitis superior, while the caudal half, running between the transverse process and the spinous process of the axis, became the obliquus capitis inferior. The rest of the muscle, running from the neural spine of the axis to the skull, disappeared. The oblique position of these muscles is due to the continued lateral expansion of the transverse processes of the atlas, which carried the attachments of these muscles out to the side. This position greatly increases the leverage of these muscles. Chapuis (1877: 296) earlier had a similar idea.

Rockwell, Evans, and Pheasant (1938: 104) have recently shown that in pronograde tetrapods the weight of the head and neck tends to produce flexion of the cervical part of the spinal column and that the

neural arches are the tension-resisting elements while the centra are the compression-resisting elements. This is shown in many long-necked, heavy-headed forms by the massive centra of the cervical vertebrae, which are in marked contrast to the smaller, weaker centra of the thoracic and even of the lumbar region. On the other hand, the cervical neural arches and neural spines are frequently more weakly constructed than the corresponding structures in other regions of the vertebral column. The comparative size and development of the neural arches and centra in a given region is thus an indication of the relative intensity of the compression and tension to which they are subjected.

Bone does not resist tension as well as ligaments do; hence most of the tension in the cervical region is resisted by the ligamentum nuchae and the ligamenta flava, both of which contain yellow elastic tissue.

The atlas-axis complex is subject to the same forces of tension and compression as are the other cervical vertebrae. The proatlas, when present, and the arch and spinous process of the atlas and axis are the tension members, while the centra are the compression members.

The head, as the result of its position at the end of the neck, is in a position of great adverse leverage and consequently its support, as well as that of the neck, is quite a problem. Support by muscles alone would be unsatisfactory, for in order to hold up the head and neck the muscles would have to be constantly contracted. This state could be maintained for only a short time and, as soon as the muscles became fatigued and relaxed, the head and neck would drop. Therefore the support of these parts has been taken over by ligaments, chiefly the ligamentum nuchae and ligamenta flava. The ligamentum nuchae is a continuation of the supraspinous ligament and is fastened to the occiput and the cervical spinous processes, while the ligamenta flava, beginning with the space between the axis and the third cervical, connects the adjacent neural arches of the vertebrae down to the last lumbar vertebra. The tension stresses created by the weight of the head and neck are transmitted by these ligaments to the spinous process and neural arch of the axis as well as to those of the other cervical vertebrae. Compression stresses in the centra of the atlas-axis complex and the other cervicals are produced by the same factor. There are no intervertebral discs in the atlas-axis complex, the first one being between the axis and the third cervical.

The axis of the higher reptiles and of mammals is characterized by a hatchet-shaped neural spine, while the mammalian atlas is distinguished by its complete ring shape, greatly expanded transverse

processes, and lack of a spinous process. All these features, as will be shown, have a functional significance.

According to Wolff's Law, the stresses to which a bone is subjected should be represented in the trabecular arrangement of the bone. This law applies to all bones and the trabecular arrangement in the vertebrae should give an indication of the stresses to which they are subjected. Gallois and Japoit (1925: 688) studied the trabecular arrangement in human vertebrae and concluded that their arrangement was in perfect accord with their function. They found three distinct systems of trabecular arrangement in the vertebrae, a principal vertical one and secondary oblique and horizontal ones. The vertical one is continuous throughout the centra as though not interrupted by the intervertebral disc. It begins in the odontoid process and ends in the sacrum. The oblique systems, two to each vertebra, run obliquely through the zygapophysis of one side to the opposite side of the centrum and vice versa. The transverse system runs from the spinous and transverse processes into the centrum. The oblique and transverse systems are not continuous from vertebra to vertebra.

The significance of these systems is that the centra are the passive elements of the spinal column and principally sustain pressure, while the neural arch and its processes are the more active elements and principally resist tension. Of course, these forces are reversed in different regions of the vertebral column and during various movements.

In the cervical region, as noted above, the centra are typically the compression-resisting elements and the neural arches, spinous processes, and ligaments the tension-resisting elements.

The odontoid is morphologically the atlas centrum and in the higher tetrapods has fused with the centrum of the axis. The vertical system of trabeculae, according to Gallois and Japoit (1925: 688), begins in the odontoid and is similar to that in the other cervical centra. This shows that the odontoid, like the centra behind it, is a compression element.

Therefore in the higher tetrapods, especially in mammals, when the atlas becomes completely ring-shaped for the rotation of the head, the odontoid must protrude through the atlas ring in order to be in a mechanical position to take up the compression stresses transmitted to the vertebral column by the skull. At the same time it forms a pivot about which the head and atlas can rotate.

It is now definitely known that muscles can influence the shape of bone and Bernhard's work on the human tibia, quoted by Murray

(1936: 78), shows that pressure causes atrophy while tension is responsible for the formation of crests. It is known also that muscles exert tension at the point of attachment to a bone.

Now the neural spine of the axis is not only subjected to the tension created by the weight of the head, transmitted to it by the ligamentum nuchae, but also to that produced by the muscles attached to it. This muscular tension is applied to the neural spine of the axis by the recti capitis muscles, the obliquus capitis muscles, and the long cervical extensors.

The tension set up by the muscles is not as constant as that due to the ligaments and is produced only when the muscles contract. Therefore, on the basis of Bernhard's work, the crest-like character of the spinous process of the axis can be explained as the result of tension stimuli produced by the muscles and ligaments attached to it. This is in keeping with the evidence that tension produces blade-like processes on bone. The formation of the neural spine of the axis between the powerful oblique and recti capitis muscles may be similar to the development of the sagittal crest between the temporal muscles.

The reduced size of the axis spinous process in man is probably correlated with the upright posture. The head is now more or less balanced on the upper end of the vertebral column so that its weight is more in line with the vertebral axis than in pronograde animals. Consequently there is only a relatively small amount of adverse leverage to resist in supporting the head and the ligamentum nuchae, the chief supporting structure of the head, is reduced, thus decreasing some of the tension on the spinous process of the axis.

The head, however, still has to be supported a little by the ligamentum nuchae, which continues to transmit tension stresses to the neural spine and arch of the axis. Although the intensity of these stresses is greatly reduced in comparison with those in a heavy-headed pronograde animal, it is almost constantly present. The tension stresses set up by the muscles are present only when the muscles contract. Therefore, in keeping with the reduced stresses to which it is subjected, the spinous process of the axis has also become reduced in size and height.

Augier, quoted in de Beer (1937: 489), has come to the conclusion, from his work on the human subject, that a process, excrescence, or crest developed by a bone arises around the point of insertion of a muscle instead of at the point of insertion. Thus the muscle lies in a depression. This conclusion is supported by the condition in some fossil forms, *e. g.*, *Diadectes*, on the occiput of which are well marked depressions for the insertion of the occipital muscles.

Augier's conclusion, in the light of Bernhard's work, would seem to indicate that the tension exerted by a muscle at its point of attachment to a bone is greater at the periphery of the muscle than at the center, since the crest is formed around the circumference of the depression.

The true significance of the proatlas has always been a knotty morphological problem and several theories, summarized by Goodrich (1930: 69) and Williston (1925: 101), have been advanced to account for it. Albrecht named it under the impression that it was the vestige of a vertebra almost completely crushed out between the skull and the atlas. Barge considered that it was the arch of the cranial half-sclerotome that forms the atlas arch. Baur held it to be the representative of a vertebra fused with the skull in reptiles, and Jaekel thought that the centrum of the proatlas was really the intercentrum of the atlas. Other theories are that it is a 'neomorph' of no special significance; that it is the vestige of the neural arch of a vertebra intercalated between the skull and the atlas, the centrum of which has either been lost or fused with the skull or odontoid; or, that it is a separated spine of the atlas.

The theory that seems most probable to me is that it is the arch of a vertebra intercalated between the skull and the atlas, the centrum of which has been lost. My reason for accepting this interpretation is based on the condition in *Sphenodon*, the most primitive living reptile, as described by Howes (1890: 358). According to him the proatlas articulates with the skull and in its relations to the epaxial musculature it repeats the conditions of the atlas. It is, like a normal vertebra, buried in the occipito-atlantar ligament of the vertebral column and its separation in the dorsal mid-line is just an exaggeration of that so characteristic of the atlas in *Sphenodon*, Crocodilia, and many other Sauropsida. Its arches are also preformed in cartilage. Its great resemblance to the atlas arch in many respects would seem to be good evidence for considering it the vestige of a neural arch between the atlas and the skull.

SUMMARY

1. A definitive neck first appeared in the Amphibia with the separation of the pectoral girdle from the skull and its later posterior migration.

2. In typical bony fish the first two vertebrae are only a little different from those succeeding them and there is no definitive atlas-axis complex. In these forms the functions performed by the atlas-

axis complex in tetrapods—namely, the attachment of the skull to the column and the providing of a central point on which the head moves, are performed by the first or first few vertebrae.

3. With the rise of the Amphibia the initial stages in the development of an atlas-axis complex occur and there are indications of a division of labor in the first two vertebrae. This is more noticeable in the primitive forms, *e. g.*, *Eryops*, in which the atlas-axis complex is quite distinct from the vertebrae behind it. The atlas is never ring-like and in the early forms the opposite halves of the neural arch and spine are separate and inclined posteriorly so as to grasp the neural spine of the axis. The neural arch, intercentrum, and centrum of the atlas have the normal relations to each other and there is a well developed proatlas between the occiput and the atlas.

4. The later fossil and the modern Amphibia are secondary, specialized forms in which the opposite halves of the neural arch of the atlas have united with each other and are fused with the centrum. The proatlas is lost, an odontoid process is developed on the cranial end of the first vertebra, the transverse process has disappeared and the spinous process is reduced to a low ridge. In modern Amphibia the first vertebra is the only cervical and rotational movements of the head occur at the occipito-vertebral joint.

5. In reptiles the tendency for the atlas to become a ring is initiated, although in most of them the opposite halves of the neural arch are still separate. The spinous process and transverse processes of the atlas are gradually reduced in size and finally disappear, while the neural arch fuses with its intercentrum. Even in the earliest reptiles, *e. g.*, *Seymouria*, the atlas centrum has moved back into the position of an odontoid, although it does not become fused with the axis until the later forms arise. The spinous process of the axis gradually increases in size and finally attains the hatchet-shape so characteristic of the higher amniotes. In modern reptiles there is some rotation of the atlas about the odontoid but there is not the perfect ring and pivot found in mammals.

6. In mammals the atlas becomes completely ring-shaped, the neural spine is lost, and large, expanded transverse processes are developed. The atlas centrum becomes completely fused with the axis to form the odontoid, about which the head and atlas rotate. The hatchet-shaped character of the neural spine of the axis is retained in typical mammals. In mammals true rotation of the head occurs at the atlanto-axial joint, while the occipito-vertebral joint serves primarily for flexion and extension movements of the head.

7. Ribs were originally present on both the atlas and the axis, but they are lost in the higher tetrapods.

8. The modern Caudata and Salientia were shown to be specialized, secondary types and not primitive.

9. The ancestral tetrapod, represented by the embolomeres, had a high skull. This type is retained by most mammals and reptiles while the later fossil Amphibia and the modern ones, except the Caecilia, have flat skulls.

10. A flat atlas-axis complex is usually associated with a flat skull. An atlas-axis complex with a high spinous process on the axis is associated with a high skull.

11. The ancestral tetrapods had a single, tripartite occipital condyle in which the basioccipital bone was the dominant element. This is essentially the condition in modern reptiles. The later Amphibia became bicondylic by the gradual loss of the basioccipital bone. The cynodonts foreshadowed the mammals by emphasizing the exoccipital portion of the condyle and became practically bicondylic functionally.

12. The mammals are completely bicondylic with separate convex condyles from which the basioccipital has been withdrawn. However, it is retained as a skull bone. The bicondylic condition was shown to be stronger than the monocondylic condition of typical reptiles. The protrusion of the odontoid through the atlas ring is necessary in order to put it in a position to take up the compression shocks coming to the vertebral column through the skull. The odontoid is shown to be a pressure resisting element by the arrangement of its trabeculae.

13. A short, phylogenetic review of the epaxial musculature from a primitive living vertebrate, *Petromyzon*, to the tetrapod stage of evolution is presented.

14. The condition and function of the occipital and cervical muscles in recent types is discussed. A similar discussion for the same muscles, as restored by the author and other workers has been presented.

15. The movements permitted by the atlas-axis complex of the various forms and the checking effects of the ligaments are discussed.

16. It has been shown that a high occiput and spinous process on the axis increased the efficiency of the occipital muscles and the amount of work they could perform.

17. The influence of muscles on the shape of bones is discussed and on that basis an explanation is offered for the characteristic hatchet-shaped spinous process of the axis in higher reptiles and in mammals.

18. An explanation is advanced to account for the origin of the transverse processes on the mammalian atlas and for the superior and inferior obliquus capitis muscles.

19. The reduced size of the neural spine of the axis in man is shown to be due to decreased tension stresses to which it is subjected.

20. The proatlas is the neural arch of a vertebra, the central elements of which are lost.

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ELECTROPHORESIS*

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INTRODUCTION TO THE CONFERENCE ON ELECTROPHORESIS

BY D. A. MACINNES

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Since the discovery, by Reuss in 1808, of electro-osmosis, a vast amount of research has been carried out in the general field of electrokinetic phenomena. The progress of discovery in the field has, however, been far from steady. Advances came about because of developments in other branches of physical science, and, more particularly, on account of the appearance of especially able investigators. As a matter of fact there was little activity in the investigation of electrokinetic phenomena until the middle of the nineteenth century. Deriving impetus from the methods and discoveries of Faraday a number of the classical researches were carried out at that time. For instance, Wiedemann made a quantitative study of electro-osmosis, and was followed by Quincke by a few years in important researches in that subject and the closely related one of streaming potentials. This active worker also made what appear to be the first experiments with electrophoresis (or cataphoresis), the subject of this conference. It was early observed that electrokinetic phenomena have considerable biological interest. Indeed the investigators were in many cases biologists, though the division of scientists into genera and species had not progressed as far as is the unfortunate state of things today. It is of interest that almost from the beginning the phenomena were interpreted in terms of what is now called the "zeta potential." It would thus appear to be a natural and useful hypothesis, although, the magnitudes and even the existence of such potentials are the subject of vigorous discussion at the present time.

By far the most important theoretical studies in the field of electrokinetic phenomena were carried out by Helmholtz. His paper, published in 1879, contains the equations used in interpreting the phenomena that are still in use today. His discussions contain, to a greater extent than is generally realized, the beginnings of the theories that have excited us in recent years. I refer especially to the concept of the diffuse double layer later developed by Gouy, and Debye and Hückel.

The other great theoretical worker in the field of electrokinetic phenomena was Smoluchowski, who, at the end of the nineteenth

century, subjected Helmholtz's ideas to a careful re-investigation, which established their essential validity.

Experimental work in the field of electrokinetic phenomena is especially difficult, due mainly to the fact that surfaces, with which the phenomena are largely concerned, are notoriously non-reproducible, making it difficult to obtain the same quantitative results in different laboratories, or even for a worker to repeat his own experiments and obtain the same figures for the results. While yet far from being as exact as some other branches of physical science much headway has been made in the last few years in the direction of the accumulation of more precise numerical data.

The conference for which the following papers are a permanent record would certainly not have been organized except for the recent appearance of the papers, by Arne Tiselius, on the study of proteins by the electrophoretic method. The utility of these methods in the whole range of biological research became immediately evident, and excited general interest in a field known, up until recently, only to a few scattered enthusiasts. Tiselius' accomplishment is, however, based, not upon a sudden or chance discovery, but upon long years of patient investigation of the phenomena of electrophoresis, and a clear understanding of the underlying electrochemistry. Because of this growth of interest and the increasing importance the subject will surely attain in the future it seemed worth while to consider the subject of electrophoresis from all the available points of view. The monograph in the reader's hand is the result of the conference that was arranged under the direction of the newly organized section of physics and chemistry of the New York Academy of Sciences.

A few words may be worth while concerning the ideas underlying the organization of the conference. It has appeared to the author and some of his associates that the older scientific societies, useful and effective as they are, fail in certain important respects. Due to crowded programs, not to mention crowded rooms, and the fact that they are in general organized around one branch of science, they fail to provide opportunities for the unhurried discussions of topics interesting to scientists representing a wide range of activities. This conference on electrophoresis was therefore arranged to provide (a) a short program of papers dealing, from widely different points of view, with the subject of electrophoresis, (b) ample time for discussion, and (c) an invited audience, limited to those capable of taking part in the discussions. It is of considerable interest that the selection of speakers and audience cut right across the usual divisions of physical

science. The conference was fortunate in bringing together mathematical physicists, physical chemists, chemists, and biologists, including those mainly interested in theory and those doing active experimental work. Although the writer had much to do with the organization, he feels free to repeat the statements of others that the conference was successful in accomplishing what it set out to do, and that it might well serve as an example to be followed, with necessary modifications, for the projected conferences under the Section of Physics and Chemistry of the New York Academy of Sciences.

THEORY OF ELECTROPHORETIC MIGRATION

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Although the first discovery of electrophoretic effects was made over a hundred years ago by Reuss¹ (1808) we have not yet a theory to explain the observed facts in a completely satisfactory manner. All electrophoretic phenomena, i. e., the migration of colloidal particles in an electric field, electro-osmosis and streaming potentials, result from the existence of electric potential differences across interphasial boundaries. The formulation of a rigorous theory of electrophoretic migration is hampered by the fact that we have very little information about the molecular structure of surfaces and about the origin of the interphasial potentials. The theory, therefore, must rely on certain hypothetical assumptions and will be subject to changes until the structure of surfaces is better understood.

The first theory of electrophoretic migration is due to Smoluchowski² (1903). It is based on the assumption, first introduced by Helmholtz³ (1879), that the potential difference is due to a very thin and rigid electric double layer. In 1909 Gouy⁴ pointed out that thermal motion does not permit the formation of a rigid layer of electric charges. His theory of the diffuse double layer was taken up later by Chapman⁵ and Stern⁶ (1924), but its full importance was realized only after Debye and Hückel⁷ (1924) furnished a broader foundation for Gouy's theory. Since 1924 the Debye-Hückel theory of strong electrolytes has played the dominant role in all questions of interphasial potentials and electrokinetic phenomena. In order to adapt it to the problems of colloidal physics it was found necessary to generalize and modify it in some points. This work has led to a much clearer understanding of many problems of electrophoretic migration, but it has not furnished the answer to all questions.

In presenting a short review of the present state of the theory it appears therefore appropriate to point out not only its successes but

¹ Reuss, F. F. *Mem. Soc. Imp. des Naturalistes de Moscou* 2: 327. 1809.

² Smoluchowski, M. *Krakauer Anz.* 183. 1903. Also in Grätz, *Handbuch d. Elektrizität u. d. Magnetismus* 2: 366.

³ Helmholtz, H. *Wied. Annalen* 7: 337. 1879. *Mem. Lond. Phys. Soc.* 1888.

⁴ Gouy, G. *Compt. Rend.* 149: 654. 1909. *J. d. phys.* 9: 457. 1910. *Ann. phys.* 7: 129. 1917.

⁵ Chapman, D. L. *Phil. Mag.* 25: 475, 654. 1909.

⁶ Stern, O. *Z. Elektrochem.* 30: 508. 1924.

⁷ Debye, P., & Hückel, E. *Physik. Z.* 24: 185, 305. 1923.

also its deficiencies. The theoretical work centers around three major problems. The first is the dynamics of electrophoretic migration, the second has to deal with the origin and structure of the electric double layer, and the last concerns the relation between migration speed of colloidal particles and the stability of colloids.

Dynamics of Electrophoretic Migration

The best known and most extensively investigated type of electrophoretic migration is that of a solid colloidal particle in an aqueous solution. We will consider here this typical example and assume the particle to be a sphere of radius R . In order that a particle can migrate under the influence of an electric field it must satisfy only one essential condition, namely, it must be at a different electrical potential than the surrounding liquid. Now Poisson's law of electro-

$$\nabla \varphi = \frac{1}{r^2} \frac{d}{dr} \left(r^2 \frac{d\varphi}{dr} \right) = \frac{1}{r} \frac{d^2}{dr^2} (r\varphi) = -\frac{4\pi\rho}{D} \quad (1)$$

statics states that a potential drop can exist only if an electric charge of density ρ is present. Equation (1) determines the distribution $\rho(r)$, of the charges in the liquid if it is known how the potential $\varphi(r)$ decreases from its value ζ at the surface ($r = R$) to the value 0 in the liquid. ζ is the electrokinetic potential. D is the dielectric constant of the liquid. Since the total charge must be zero the "outer" charges in the liquid are compensated by a charge Q of the particle, whereby, according to Equation (1),

$$Q = - \int_0^\infty \rho \, 4\pi r^2 dr = \int_R^\infty D \frac{d}{dr} \left(r^2 \frac{d\varphi}{dr} \right) dr = -DR^2 \left(\frac{d\varphi}{dr} \right)_{r=R} \quad (2)$$

A priori, this "inner" charge Q might be located anywhere within the particle, but it is generally assumed that it is spread in a layer over the surface. The inner charge Q and the outer charge distribution ρ are of opposite signs and are said to form an electric double layer. Helmholtz assumed that the outer charges were concentrated in a single layer at a definite distance t from the surface. This thickness t of the double layer was assumed to be of atomic dimensions (10^{-7} cm). The Gouy-Debye theory shows that the outer layer consists of an ionic atmosphere in which the charge density ρ and the potential φ decrease gradually from a large value near the surface and extend over a range of about 10^{-4} cm. in dilute solutions. The thickness of such a diffuse double layer is defined as that distance t of separation of an

equivalent Helmholtz layer of charge Q which would produce the same potential drop as exists in the diffuse layer.

If the outer charges were missing the velocity of the particle in an electric field X would be given by Stokes' law

$$u = \frac{QX}{6\pi\eta R} \quad (3)$$

This law takes into account only the viscous resistance of the hydrodynamic currents which are created by the motion of the particle. The presence of the outer charges alters these currents because these charges are also influenced by the electric field. They move in the direction opposite to that of the particle. They create therefore an additional counter-current of the surrounding liquid and thus the particle meets an increased viscous resistance. Hence the velocity of the particle will not be u but

$$V = u + v \quad (4)$$

The (negative) change of velocity v which is due to "electric friction" can be estimated as follows. Consider within the liquid a thin spherical shell of radius r and thickness dr . This shell contains a portion $dQ = 4\pi r^2 \rho dr$ of the outer charge. If dQ were the only charge present the shell and its interior would gain a velocity

$$dv = \frac{XdQ}{6\pi r\eta} = \frac{4\pi r\rho Xdr}{6\pi\eta}$$

The additional electrophoretic current of the liquid can therefore be found if one takes into account that each moving shell imparts its velocity to its inner neighbor. A shell at a large distance ($r = \infty$, $\rho = 0$) stays at rest, while the innermost shell has a velocity equal to the sum of all increments dv ; hence

$$v = \int_R^\infty dv = -\frac{DX}{6\pi\eta} \int_R^\infty \frac{d^2}{dr^2}(r\varphi) dr = \frac{DX}{6\pi\eta} \frac{d}{dr}(r\varphi)_{r=R}$$

In the above integration we have made use of Equation (1). The innermost shell is assumed to adhere to the particle and impart to it the same additional velocity v . The above result can also be written

$$v = \frac{DX}{6\pi\eta} \left[R \left(\frac{d\varphi}{dr} \right)_R + \varphi(R) \right] = -u + \frac{DX\zeta}{6\pi\eta}$$

because it follows from Equation (2) that $R \left(\frac{\partial\varphi}{\partial r} \right)_R = -\frac{Q}{DR}$ and hence

the first term has the same magnitude as u defined in Equation (3). By introducing this result in Equation (4) we find for the electrophoretic migration speed

$$V = u + v = \frac{DX\zeta}{6\pi\eta} \quad (5)$$

This is the electrophoretic migration formula of Debye and Hückel.⁸ The above derivation is due to Onsager.⁹ According to this equation the migration speed is determined by the electro-kinetic potential ζ and does not depend on the charge distribution within the double layer. It should therefore be valid for a rigid double layer as well as for a diffuse one. If this result were correct it would be impossible to decide from electrophoretic measurements whether the Helmholtz or Gouy theory is applicable.

There are, however, many reasons to believe that the above equation is not generally valid. It differs from the result of Smoluchowski's theory, which gives for an arbitrary shape of the particle

$$V = \frac{DX\zeta}{4\pi\eta} \quad (5')$$

According to Hückel this equation should be correct only for needle-shaped particles or for electro-osmosis in capillaries, while for other particles the numerical factor should depend on the shape of the micelles. From the work of Abramson¹⁰ we know that this conclusion does not agree with the facts. Particles of different shapes have the same migration speed if they have identical surfaces and if the double layer is thin in comparison with the dimensions of the particles.

Mooney¹¹ and Henry¹² have pointed out the deficiency of the Debye-Hückel equation. In the above derivation we have assumed that the electric field X is everywhere constant, and that all parts of any spherical shell could move with the same velocity in the same direction. This assumption is wrong because the presence of the particle forces the electrical and the hydrodynamical currents to flow around the micelle. These effects, due to the finite size of the particle, can be neglected only if the micelles are very small in comparison to the thickness of double layer. The Debye equation (5) is therefore valid only in the very rare cases of extremely small particles in very dilute

⁸ Debye, P., & Hückel, E. *Physik. Z.* **25**: 49, 204. 1924.

⁹ Onsager, L. *Physik. Z.* **27**: 388. 1926.

¹⁰ Abramson, H. A. *Jour. Phys. Chem.* **35**: 299. 1931. Also 'Electrokinetic Phenomena' Chem. Catalog Co. 1934.

¹¹ Mooney, M. *Jour. Phys. Chem.* **35**: 331. 1931.

¹² Henry, D. C. *Proc. Roy. Soc. (London)* **A. 133**: 106. 1931.

solutions. For the other limiting case, where the range of the double layer is small in comparison to the radius of the particle, Henry¹² has derived the equation

$$V = \frac{(1 + \lambda) D X \zeta}{6 \pi \eta} \quad (6)$$

where λ depends on the electrical conductivity of the micelle.

For non-conducting particles λ has the value $\frac{1}{2}$ and Henry therefore comes to the conclusion that Smoluchowski's equation (5') should be valid in most cases. Deviations from this equation must occur if the radius of the particle is of the same order of magnitude as the thickness of the double layer. In this case the numerical factor will vary between 4 and 6; it will depend on the charge and on the shape of the particle. For conducting particles Henry's equation (6) is probably not valid.

Smoluchowski's, Debye and Hückel's, and Henry's derivations of the migration formula are based on the assumption that the motion of the particle does not alter the charge distribution in the double layer. For dielectric micelles there is no reason to expect a change of the "inner" charges. For conducting particles, however, the electric current might alter the conditions on the surface. The outer diffuse layer will always be affected by the hydrodynamic flow. During the migration of the particle this flow brings new ions into the neighborhood of the surface and carries other ions away from the micelle. Thus the outer layer is continually destroyed and rebuilt. The new ions require a certain time to adjust their distribution to the electric field distribution, and the ions which are carried away retain their distribution for a short time. Due to this retardation effect the outer layer becomes asymmetric and exerts an electric force on the inner charges which reduces the migration speed of the particle. Paine¹³ (1932) and more recently Hermans (1938) have calculated the magnitude of the relaxation effect. In most cases it is small, but in his latest paper Hermans¹⁴ finds that under favorable conditions the effect can alter the migration velocity by as much as 25%.

It is partly due to this retardation effect and partly due to the fact that Smoluchowski's equation contains the dielectric constant D and the viscosity η of the liquid, that the theorist is reluctant in accepting it as a rigorous solution of the problem. The use of the macroscopic constants D and η has frequently been criticized but no satisfactory alternative has been given. Smoluchowski's equation is better

¹³ Paine, H. H. Proc. Cambridge Phil. Soc. 28: 83. 1932.

¹⁴ Hermans, J. J. Phil. Mag. 25: 426. 1938. 26: 650. 1938.

justified than any other, but it is questionable whether the absolute values of the ζ potentials, as obtained with the help of this equation from migration speed measurements, are accurate.

The Electric Double Layer

While the existence of electric double layers in interphases between solids and liquids can be taken as an established fact, very little is known about the causes for its existence. We can easily understand how the outer layer is formed when the solid surface carries a charge, but the primary question: Why and how is the solid charged? is much more puzzling. Not only is it most improbable that a single answer could be given which would fit all cases, but there are very few interphases for which any one of the proposed answers has been generally accepted. In most cases we have good evidence that these surface charges are ions, or that the colloidal particle themselves are ions, but it does not seem impossible that in some cases the charge is due to a transfer of electrons from the solid to the liquid, or vice versa. It is not definitely known what forces are responsible for holding the ions to the solid. At various times they have been assumed to be Van der Waals forces (adsorption), valency forces (chemical binding) or lattice forces¹⁵ (theory of potential determining ions). The recent progress of the theory of the electronic structure in metals and insulators and of the theory of liquids opens a good prospect that a more specific answer can soon be given.

Various methods have been used to estimate the electric charge density in the inner layer. All lead to the conclusion that the average distance between the "adsorbed" ions is about 10^{-6} to 10^{-7} cm., i. e., less than 1% of the atoms on the surface are charged. This distance is only about 10 times smaller than the average thickness of the double layer. Consequently the double layer can not be as homogeneous as we usually like to assume. The potential varies along the surface and what one measures in the experiments is an average value. This average can be considerably smaller than the potential of the "adsorbed" ions. It is therefore not surprising that the Nernst potential ϵ , which measures the work needed for removing an ion from the surface, is always much larger than ζ . This argument, however, is not sufficient to explain the fact that ϵ and ζ can have different signs.

Before the advent of the theory of the diffuse double layer and Debye's theory of strong electrolytes, all changes of the potential were ascribed to changes of the "adsorbed" charges. The newer theory can

¹⁵ Verwey, E. W. J. Symposium on Hydrophobic Colloids. Centen's, Amsterdam, 1938.

explain a large part of the potential variations as due to changes in the outer layer. But they do not explain all variations and we must assume that the concentration of the surrounding electrolyte influences the magnitude and sign of the inner charges.

Debye's theory explains the formation of the outer layer as follows: If the charge of the inner layer is positive, the negative ions in the liquid are attracted to the surface and the positive ions are repelled. Thus a predominantly negative "ionic atmosphere" is created. The outer charges do not form a single layer, as Helmholtz had assumed, because the energy $ze\phi$ of attraction or repulsion usually is smaller than the kinetic energy kT of the ions. This is certainly true in the outer parts of the outer layer where the potential ϕ approaches the value 0. In the range where $ze\phi$ is not much larger than kT the ions can move freely, but the negative ones linger for a longer time near the surface than do the positive ions. The calculation of the charge distribution in the diffuse double layer is therefore a statistical problem. Its solution is obtained by using Maxwell-Boltzmann's principle which gives the distribution of charges in a potential field. This principle furnishes therefore a second relation between ϕ and ρ , the first being Poisson's equation (1). The two relations permit the calculation of potential and charge distribution in the outer layer.

The theory of the diffuse double layer leads to the conclusion that the effective thickness of the diffuse double layer and hence also the ζ potential diminish if the concentration c of the electrolyte is increased. For very small concentrations the outer layer must quite obviously be very thick to be able to compensate the charges on the particle. In certain selected cases it is possible to account for the observed $\zeta(c)$ curves by assuming that the charge of the particle is unchanged and that the variations are due only to changes of the outer diffuse layer. No calculations of this type are really convincing because they involve the assumption that the absolute values of ζ are known. They prove, however, that the changes of the outer layer have a great influence on the ζ potential.

The second satisfactory feature of this theory deals with the influence of the valency of the ions on the ζ potential. The larger the ionic charges the larger are the electric forces between them and the inner layer and the fewer ions are needed for charge compensation. Hence the effective thickness of the double layer and with it the ζ potential diminish rapidly as the valencies increase. The experiments confirm this general conclusion, but they also show that the approximations of Debye's theory are not valid for colloids. Debye and Hückel assumed

that the electric potential energy $ze\phi$ is always smaller than their kinetic energy kT . If this were true for the ions in the double layer, the ζ potential should follow the ionic strength principle. This principle states, for instance, that a 1-3 valent electrolyte should have the same effect on the potential as a 3-1 valent electrolyte. The experiments do not verify this. This fact is of importance because it proves that the ζ potentials as calculated from Smoluchowski's equation are of the correct order of magnitude. For $z > 1$ their values are indeed so large that $ze\zeta$ is usually greater than kT and Debye's approximation can not be expected to hold. If in the equation $V = \frac{DX\zeta}{C\eta}$ the factor

C would be appreciably smaller than Smoluchowski's 4π it would be difficult to understand the failure of the ionic strength principle.

The differential equation of the diffuse double layer can be solved without resorting to Debye's approximation. The first solution was given by Gouy for a plane surface, the writer¹⁶ (1928) developed a graphical solution for a spherical double layer and Gronwall, La Mer and Sandved¹⁷ (1929) gave an analytical solution. This work predicts deviations from the ionic strength principle which are in good qualitative agreement with the observations.¹⁸ This fact offers some justification for applying the laws of the diffuse double layer not only to its outermost parts but also to its inner range where ζ approaches its maximum value ϕ . On the other hand, it must be pointed out that this extrapolation may have little justification. In the immediate neighborhood of the solid surface the physical conditions are extremely complicated, the electric field strengths become very high, the ordinary macroscopic concepts have no definite meaning, and the fundamental assumptions of Debye's theory (complete dissociation) are likely to break down. The structure of this innermost layers is a matter of pure speculation. The most commonly accepted view is that of Stern,⁹ who assumes the existence of an intermediate layer between the particle and the diffuse layer. This Stern-layer adheres to the particle. In Stern's theory the ζ potential is therefore not the average potential of the particle but that on the outer boundary of the Stern layer. This assumption serves to explain the difference between the ϵ and ζ potentials, but there is no direct experimental evidence for the existence of this intermediate layer.

In summarizing it may be said that the theory of the diffuse double

¹⁶ Mueller, H. *Kolloidchem. Beihefte* 26: 257. 1928. Cold Spring Harbor Symposia 1: 1, 9. 1933.

¹⁷ Gronwall, T. H., La Mer, V. K., & Sandved, K. *Physik. Z.* 29: 358. 1928.

¹⁸ See discussion on Colloidal Electrolytes. *Trans. Faraday Soc.* 31: 1935.

layer, though it has contributed a great deal to the theory of electrophoretic migration, is not able to predict the changes of the migration speed with variations of the electrolyte concentration. A theory of these changes can only be given when we know more about the origin of the "adsorbed" charges. A solution of this problem may solve also the questions concerning the structure of the immediate neighborhood of the surface.

The Stability of Hydrophobic Colloids

It is well known that the stability of a hydrophobic colloid is closely related to the migration speed of its micelles. Coagulation usually sets in when the ζ potential drops below a critical value, but there are exceptions to this rule. On the other hand, the coagulation of a hydrophobic colloid is not reversible, i. e., the coagulate is not peptised through an increase of the ζ potential. This behavior is usually explained as follows. Uncharged particles would always adhere because they are attracted by Van der Waals forces. Since these forces have a very short range the particles have to come very close together before they attract each other. The electric charges of the particles and of their double layers create repulsive forces between the micelles. These electric forces have a larger range than the Van der Waals forces, and their intensity is supposed to increase with the ζ potential. Therefore, if the ζ potential is high the particles can never approach each other sufficiently closely to come under the influence of the attractive forces, and the colloid is stable. The electric forces constitute a potential barrier. For low ζ potentials this barrier is low and the particles have sufficient kinetic energy to cross it. Hence they will coagulate under the influence of the attractive Van der Waals forces. If afterwards the barrier would be raised again by an increase of the ζ potential, the chances for a separation of the particles are even smaller than before when the barrier was low.

Hamaker¹⁹ (1936) has calculated the Van der Waals forces and has given an estimate of the electrical forces between colloidal particles. His conclusions, which support the above qualitative discussion, have recently been challenged by Langmuir²⁰ (1938). A somewhat different point of view is contained in a paper by Levine.²¹ This writer has made a more elaborate calculation of the electrical forces between the diffuse double layers of two colloidal particles. He finds that they

¹⁹ Hamaker, H. C. Symposium on Hydrophobic Colloids. *Rec. trav. chim.* **57**: 61. 1938.

²⁰ Langmuir, I. *Jour. Chem. Phys.* **6**: 873. 1938.

²¹ Levine, S. *Proc. Roy. Soc. (London)* **A941**: 145. 1939. The author had the privilege of reading this manuscript before its publication.

lead to an attraction at large separation. However, if the particles come at a distance smaller than the thickness of the double layer, this force becomes a repulsion and creates stability of the colloid in a similar way, as in Hamaker's theory. The attraction of the particles at large distance of separation comes about as follows. The total electrical force between the two particles can be considered as the combination of four forces: a) A repulsion between the adsorbed charges on the two particles, b) a repulsion between the charges in the two diffuse layers, and c) two attractive forces between the inner charges of one particle and the outer charges of the other. For very large separation the resultant of all four forces must be zero; with a closer approach all forces will increase but when the two double layers begin to overlap the repulsive force (b) will increase less than the other forces and hence the balance of the forces is disturbed and gives rise to an attraction. For a still closer approach, when the inner charge of one particle penetrates the double layer of the other the attractive forces (c) are strongly diminished, and the resultant becomes a repulsive force.

According to Levine's theory, the colloidal particles should have a preferred distance of separation. Usually they can not stay in this preferred position, due to the temperature motion, but under exceptional circumstances they can assume a regular arrangement. These considerations may possibly lead to an understanding of the formation of "tactoids" and the structure of "thixotropic" colloids.

We have left out in these discussions the interaction between the micelles and the solvent. These interactions are certainly of great importance in lyophilic colloid. It is questionable whether they can be neglected in the hydrophobic sols. We evidently have still a long way to go before we know all the factors which determine the behavior of colloids and can give a satisfactory theory of the electro-kinetic phenomena.

THE DETERMINATION OF THE ELECTRICAL CHARGE OF SURFACES BY THE MICROSCOPIC METHOD OF ELECTROPHORESIS

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INTRODUCTION

With few exceptions the surfaces of all particles suspended in aqueous media bear an excess of negative or positive electricity. For this reason, red blood cells, bacteria, spirochetes, oil droplets, particles of quartz, protein and even air bubbles almost invariably migrate if an electric field of sufficient strength is applied. The movement of these particles is readily observed and measured by means of ordinary microscopic equipment. The speeds of fairly large oil droplets, surprisingly enough, may be greater than those of ordinary ions. For example, oil droplets 1×10^{-3} cm in radius suspended in sodium hydroxide solution have electric mobilities *faster than sodium ions*.¹ It is remarkable to observe directly, microscopic particles which move with high speeds in comparatively low electric fields of 5 to 10 volts per cm. In a field of 10 volts per cm, charged oil droplets can cover distances of 1.0 cm in less than two minutes. Two quantities of importance in the comprehension of the phenomena of surface chemistry and surface electrification may be calculated from the electric mobilities measured by the microscopic method of electrophoresis. The first is the electrokinetic potential, ζ ; the second is the net charge density, σ , of the surface.

According to the Helmholtz theory, the dependence of ζ on the mobility v is given by the expression

$$\zeta = \frac{4\pi\eta}{D}v \quad (1)$$

where η , the coefficient of viscosity, and D , the dielectric constant of the double layer, approximate their values in the bulk of the liquids.² Units are c.g.s. electrostatic.

¹ Abramson, H. A. *Am. Jour. Physiol.* **97**, 1931.

² Smoluchowski, M. "Handbuch der Electricität und des Magnetismus," Barth, Leipzig 2: 366, 1921.

Note that σ , the density of the electric charge of the surface, does not appear in equation (1). Evidently if our study of surface electrification is to be more complete, the relationship between the charge density and the mobility must also be known. Fortunately this relationship is not only known but also, by the application of this theory, important generalizations may be made concerning the way the charging process of non-ionogenic surfaces varies with change in the salt content of the medium.³

If the concentration of an aqueous solution of potassium chloride is increased, the electric mobilities of the ions, K^+ and Cl^- , decrease. This typical decrease in the ionic electric mobility, v , is depicted in figure 1, where the change in v_{K^+} with the molar concentration, c , of potassium chloride is given. Compare FIGURE 1 with FIGURE 2 which

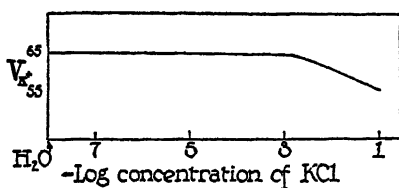


FIGURE 1. Schema giving change in the electric mobility of an ion with increasing σ of increasing concentration of the electrolyte.

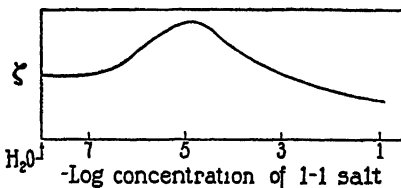


FIGURE 2. Typical curve showing effect on ζ of increasing the concentration of a simple salt.

is a typical ζ - c or v - c curve. Note that a maximum occurs before the decrease in mobility due to the addition of salt. The case of ionic electric mobility in FIGURE 1 represents the ideal case in which the charge remains constant while the electric mobility changes.

In the cases of non-ionogenic surfaces the problem becomes more general in nature, for we must deal with particles having diameters from the order of 1×10^{-7} cm to those of the order of macroscopically visible particles, including flat surfaces. The charge of the simple ion K^+ , as has been mentioned, remains constant as v_{K^+} decreases. This is by no means the case with non-ionogenic surfaces.⁴ The net

³ Abramson, H. A. Jour. Phys. Chem. 39: 749. 1935.

⁴ Gouy, L. Jour. Phys. 9: 457. 1910.

Gyemant, A. "Grundzuge der Kolloidphysik" Vieweg, Braunschweig. 75. 1925.

Mueller, H. Kolloidchem. Beih. 26: 257. 1928.

Mooney, M. Jour. Phys. Chem. 35: 331. 1931.

Abramson, H. A., & Mueller, H. Bull. Am. Phys. Soc. 7: 11. 1932. Cold Spring Harbor Symposium 1. 1933.

Bikerman, J. Z. physik. Chem. A163: 378. 1933.

By non-ionogenic surfaces is meant surfaces like quartz, paraffin oil, cellulose, etc. By ionogenic surfaces is meant those resembling adsorbed protein films. Most surfaces, undoubtedly, behave to a certain extent in an intermediate fashion, but this classification will suit the purposes of this communication.

surface charge density, σ , changes as more ions are added to the solution. In general, for an infinite plane surface, σ can be calculated by combining the Poisson and Boltzmann equations. The integrated equation for σ , is, in systems consisting essentially of two ions and large particles,⁴

$$\sigma = \sqrt{\frac{NDkT}{2\pi \times 1000}} c_{1[2]} z_{1[2]} \left[\frac{1}{z_2} \left(e^{+z_2 \frac{e\zeta}{kT}} - 1 \right) + \frac{1}{z_1} \left(e^{-z_1 \frac{e\zeta}{kT}} - 1 \right) \right] \quad (2)$$

where N is Avogadro's number, k Boltzmann's constant, c the molar concentration, e the base of the natural logarithm, e the electronic charge, z the valence, and T the absolute temperature. In case $z_1 = -z_2 = z$ in aqueous media, equation (2) reduces to

$$\sigma = 2 \sqrt{\frac{NDkT}{2\pi \times 1000}} \sqrt{c} \sinh z \frac{e\zeta}{kT}, \quad (2a)$$

or,

$$\sigma = 2\alpha \sqrt{c} \sinh \frac{z\zeta}{2\beta}.$$

where $\alpha = 17,650$ and $\beta = 0.025$ volt at 18°C . Equations (2) and (2a) give us a means of following changes in the net surface charge of a large particle as the number of ions in the solution increases.

THE MEASUREMENT OF ELECTROPHORETIC MOBILITY OF MICROSCOPIC PARTICLES

Historical

Although Reuss discovered the phenomenon of electrophoresis in 1807, investigators refrained from completely accepting the fact that a new series of electrical phenomena were taking place on surfaces, until Quincke's ingenious experimentation on the transport of microscopically visible particles suspended in liquids was published. Quincke⁵ studied the electrical mobility of microscopic particles in types of ap-



FIGURE 3. Particles or gas bubbles were suspended in the narrowed portions of the tubes here depicted and a field applied to study electrophoresis (Quincke).

paratus similar to those shown in FIGURE 3. Very small particles

⁴ Quincke, G. Pogg. Ann. 113: 513. 1861.

were suspended in the tube and even air bubbles were introduced by micropipettes. By one device or another tiny particles of many substances were investigated. All of these particles were negatively charged in distilled water. In other media similar particles were found to be positively charged as well. Quincke's observations are not only of importance because they generalize the phenomenon of electrophoresis but also because he observed and correctly interpreted certain hydrodynamic features of the electrophoresis cells which he employed and which we shall discuss in detail. Quincke noticed that the movement of the particles in the electrophoresis cell was not alone due to the electrical charge of the particles but also to the movement of the liquid within the cell itself. Quincke noted that starch granules in the center of the electrophoresis cell migrated to the anode but that particles adjacent to the glass wall went in the opposite direction, to the cathode. Quincke recognized this reversal of migration as due to an electroosmotic flow of liquid near the glass. He states, "It seems to follow from these experiments if one takes the representative case of starch particles suspended in water, that water is transported in the direction of positive flow of electricity and all starch granules in the negative direction. The transported water (since the cell is a closed system) returns along the axis of the cell for it can go nowhere else. Near the wall of the tube the water drags the starch granules along with it and overcomes the mobility of the granule, while in the neighborhood of the axis the mobility of the starch particles will be increased by the movement of the water particles." As we shall see shortly the electroosmotic flow of liquid in electrophoresis cells designed for the study of microscopic particles is nearly always complicated by the electroosmotic flow of liquid. However, this hydrodynamic problem is readily solved and a suitable correction may be made.

Scope of the Microscopic Method

Whenever a particle is observable in the field of a microscope or untra-microscope, the microscopic method of electrophoresis may be conveniently and accurately employed if the particle does not settle out of suspension too quickly.⁶ The ease of the microscopic method offers certain advantages when compared with the moving boundary method. However, the moving boundary method is essentially designed to study dissolved material,⁷ whereas the microscopic method

⁶ Abramson, H. A. *Jour. Phys. Chem.* **36**: 1454, 1932.

⁷ Tiselius, A. *Dissertation*, Upsala, 1930.

Henry and Brittain recently have compared critically the moving boundary method with the transport method. "The Kohlrausch-Weber theory of ionic displacements has

is designed for the study of particles. The two methods supplement one another. The microscopic method offers in part the following advantages:

a—During the period of observation the environment of the particle does not change sensibly.

b—Differences in electric mobility of various particles in the same microscopic field may be measured simultaneously.

c—High magnification may be employed resulting in great sensitivity.

d—The size, shape, and orientation of the particle under observation are directly observed.

e—Measurements of electric mobility may not only be made in fairly concentrated salt solution (up to about M/5) but also in very dilute solution. As far as the writer is aware, measurements by the moving boundary method may not be made without encountering serious difficulties except in the presence of at least M/100 salt.⁷

f—A single determination usually takes only a few minutes.

g—The method is the only way in which certain biological material may be accurately studied. Thus bacteria, fungi, blood cells, protein particles are most easily examined in this way.

h—There is no difficulty in calculating, from the curve relating electric mobility to depth in the cell, the electroosmotic mobility of the liquid relative to the wall of the cell itself.

i—By means of the vertical cell⁸ the same particle may be observed over a comparatively long period. Changes in the surface of the particle during this time may be observed.

The Flat Cell in Rectangular Cross Section

Two flat cells recommended by the writer are shown in FIGURES 4 and 5. FIGURE 4 is a flat cell⁹ which is used in horizontal position, while FIGURE 5 is a cell used with a horizontal microscope, the chamber of the cell being fixed vertically.⁸ The instruments, each of which is

been applied to the moving-boundary method, and it is inferred that the usual interpretation of experiments by this method is erroneous. Methods of experiment and calculation are, however, indicated by means of which correct results may be obtained by the moving boundary technique, and some conclusions are drawn as to the selection of a suitable supernatant liquid." (*Trans. Farad. Soc.* 29: 798, 1933.) This paper should be consulted by those intending to investigate theoretically or experimentally the methods under discussion since these new contributions have not been incorporated here. See also *Hacker. Kolloid-Z.* 62: 37, 1933.

⁷ Abramson, H. A., Moyer, L. S., & Voet, A. *Jour. Am. Chem. Soc.* 58: 2362, 1936.

⁸ Abramson, H. A. *Jour. Gen. Physiol.* 12: 469, 1929.

Abramson, H. A., & Grossman, E. B. *Jour. Gen. Physiol.* 14: 563, 1931.

Moyer, L. S. *Jour. Bact.* 31: 531, 1936.

constructed of one piece of glass, rest directly on the microscope stage or are suspended from it. In this way the troublesome vibration usually encountered may almost completely be avoided. The instrument may be supported either by a cemented frame composed of microscope slides or by a brass frame similar to that shown in FIGURE 4 and described by Moyer. The vertical cell shown in FIGURE 5

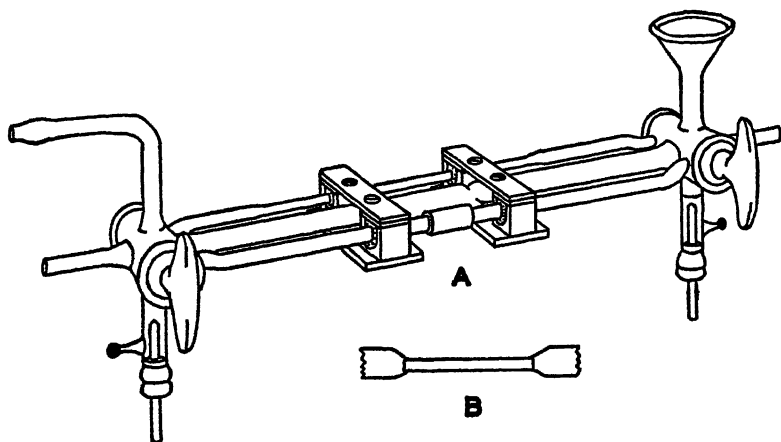


FIGURE 4 The horizontal microelectrophoresis instrument The smaller rods on either side of the center chamber are solid glass struts, one with an extension sleeve. B, Diagram of side view of central chamber.

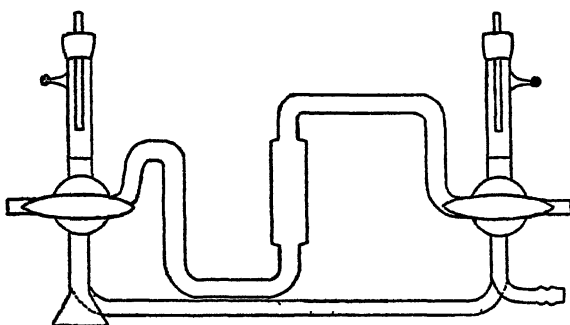


FIGURE 5. The vertical microelectric cell.

facilitates the investigation of large particles for long periods of time by reversal of the electric field. Because of its vertical position, the particles, although falling, remain at the same electroosmotic level. If the width of the main central chamber is very large compared with its thickness the effect of the size of the cell may be disregarded when observations are not made too close to the side walls. The electric

mobility of the particle relative to the liquid may be calculated as was first accomplished by Ellis.¹⁰ Ellis observed quantitatively the variation in the electric mobility of small oil droplets at different levels in a flat electrophoresis cell, approximately 0.6 mm thick. His data are plotted in FIGURE 6 which shows in detail the phenomenon of reversal of direction of the particle near the wall observed first by Quincke and described in the first part of this section. Observe in the figure that the velocity of a small particle is not constant but varies from level to level within the cell, the velocity-level curve being parabolic. The velocity of the particle may be increased or decreased at any point, depending upon the sign of the charge on the wall. If V_w is the velocity of the liquid itself at any level, the observed velocity V_{OBS} is, then, the sum of the velocities of the particle and of the water.

$$V_{OBS} = V + V_w \quad (3)$$

Here V is the velocity of the particle relative to the liquid; it is the same at all levels. In other words, since the velocity of the particle relative to the liquid is the same at all levels, V_{OBS} varies from level to level because of the hydrodynamic conditions set up by the streaming of the liquid within the cell due to the electroosmosis of the liquid along the wall. It can be shown that the velocity of the particle relative to the liquid is the mean velocity of the particle within the cell, if the total movement of all the water within the cell is equal to zero. The value of V , the electric mobility of the particle relative to the liquid, can be calculated from experimental data relating V_{OBS} to the level, x , in the cell, or by means of graphical integration. Also, measurements may be made simply at the level,

$$x = d \left(\frac{1}{2} \pm \frac{1}{\sqrt{12}} \right), \quad (4)$$

where d is the total thickness of the cell. The stationary levels in cells of the type under discussion are approximately at the levels 0.2 and 0.8 of the total depth. This follows from the theory of Smoluchowski² regarding the laws of laminal streaming of viscous liquid between parallel walls. He showed that the velocity of the water, V_w at a depth, x , is

$$V_w = U \left[1 - 6 \left\{ \frac{x}{d} - \left(\frac{x}{d} \right)^2 \right\} \right], \quad (5)$$

where U is the velocity of electroosmosis due to the charge on the wall

¹⁰ Ellis, R. Z. physik. Chem. 78: 321, 1912.

of the cell. It follows directly from equation (5) that when V_W is equal to zero, the velocity of the particle relative to the liquid may be measured directly. The arrow in FIGURE 6 points to both the observed

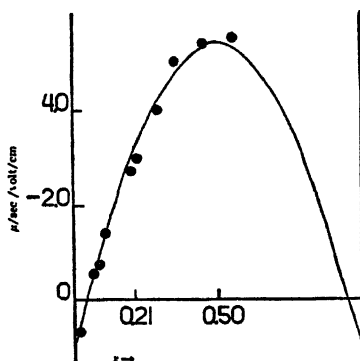


FIGURE 6. The variation in the observed electric mobilities of oil droplets at different levels in a flat electrophoresis cell. The smooth curve is that predicted by hydrodynamic theory. Points are experimental values found by Ellis who first investigated this phenome-

and predicted values of velocity given by the preceding theory and agrees with the theory well within 5%. The reliability of this theory¹¹ has been well established for the types of cells given in FIGURES 4 and 5. The way in which variations in the thickness of the cell change the the general shape of the experimental curve is given in FIGURE 7.

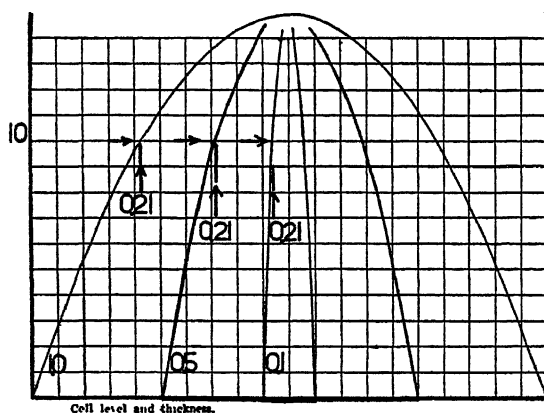


FIGURE 7. The way in which variation of the thickness of an electrophoresis cell (width \gg thickness) changes the experimental curve. The curves have been drawn on the assumption that $V = U = 1.0$, for cells varying in thickness, $1.0 : 0.5 : 0.1$.

¹¹ Abramson, H. A. "Electrokinetic Phenomena and their Application to Biology and Medicine." Chemical Catalog Co., New York, Chap. 3, 1934.

The curves have been drawn on the assumption that the electrophoretic velocity is equal to the electroosmotic velocity. Note that the slope of the $V_{OBS} - x$ curve at the level where the velocity of liquid is equal to zero, becomes very great with thin cells. Practically speaking then, flat cells less than 0.5 mm thick may involve difficulties in focusing at a given level.

The theory of Smoluchowski obtains only if the cell width is great compared with its thickness. If the ratio of cell width to thickness is taken as,

$$\frac{\text{width}}{\text{thickness}} = \frac{2a}{2b} = k,$$

Komagata¹² finds that the position of the stationary layer along the central longitudinal plane is given by the expression,

$$y_{(V_W=0)} = \pm b \sqrt{\frac{1}{3} \left(1 + \frac{384}{\pi^2 k} \right)}$$

In this equation y represents the level measured from the central axis. The following table¹¹ indicates the error introduced for various values of the ratio a/b .

k	10	15	20	100	∞
level	0.194	0.199	0.202	0.210	0.211

Note that only when k approaches ∞ does the stationary layer correspond exactly to our previous stationary level, about 0.21 measured from the roof. Flat cells, with values of k more than 20, may be used, without considering the wall effect.

Since the curve relating the observed velocity to level is parabolic, an equation of the form

$$V_{OBS} = m(x - x^2) + C \quad (5)$$

may be used to obtain a linear relationship between the observed velocity and the depth.¹³ This allows the particle velocity at the ceiling or floor of the cell to be obtained by linear extrapolations to the point $x = 0$. The electroosmotic velocity¹³ may then be calculated directly by equation (5), for at the wall,

$$V_{OBS} = V + U$$

¹² Komagata, S. "Researches of the Electrotechnical Laboratory." Tokyo. 1933.

¹³ Moyer, L. S., & Abramson, H. A. Jour. Gen. Physiol. 19: 727. 1936.

The Cylindrical Cell of Circular Cross Section

Types of cells of cylindrical cross section¹⁴ are illustrated in FIGURE 8. Although hydrodynamically these cells are apparently more suit-

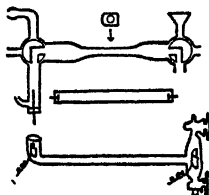


FIGURE 8. Types of construction of electrophoresis cells of circular cross section.

able than the flat electrophoresis cell, there are certain optical difficulties which at present make their use more complicated. There is, for example, considerable distortion of the images of objects suspended in cells of this type unless special optical precautions are taken. Focusing along the diameter becomes more difficult as the distance from the roof of the cell is increased. The observed velocity of a particle is, similar to the case of the flat cell, equal to

$$V_{OBS} = V + V_w,$$

which in the cylindrical cell can be shown to be,

$$V_{OBS} = V + U \left(\frac{2a^2}{r^2} - 1 \right)$$

where r is the radius and a the distance from the axis. It is our problem to find the value of r in terms of a when V_w is zero, that is when

$$U \left(\frac{2a}{r^2} - 1 \right) = 0,$$

or

$$a = \frac{r}{\sqrt{2}},$$

the position of the stationary level is obtained. The flow of liquid is zero in a cell of circular cross section at a distance of $\frac{r}{\sqrt{2}}$ from the axis or 0.14 of the cell's diameter measured from the roof of the cell. This corresponds to the level 0.21 for the flat cell. It is advisable in the

¹⁴ Mattson, S. Kolloidchem. Beih. 14: 227. 1922. Jour. Phys. Chem. 32: 1532. 1928. 37: 223. 1933.

Mooney, M. Phys. Rev. 23: 396. 1924.

case of the cylindrical cell to plot the $V_{OBS} - a$ curve to obtain a linear relationship for the velocity of the particle relative to the liquid using the expression,

$$V = \frac{1}{r^2} \int_0^r V_{OBS} d(a^2).$$

The value of this integral should be equal to the value of V at the level 0.14.

In connection with the difficulties in focusing, it has been shown by Boswell and Larson¹⁵ that the use of cylindrical electrophoresis cells is complicated by optical phenomena encountered in ascertaining the stationary level. If the lighting is from one side, a beam entering at the stationary level will be bent upward to the extent of 0.195 of the radius from the top and hence will give an erroneous measurement if the microscope is focused on the beam. A recent paper by Henry¹⁶ discusses this error in focusing in more detail.

Calculation of Potential Gradient

In the event that X , the potential gradient, is not simply the applied e.m.f. divided by the length of the cell, several methods are available for calculating X .⁹ It may be determined from the dimensions of the entire system, from a calibration of the system with mercury, by means of sealed platinum electrodes which measure the potential difference with the aid of an electrostatic voltmeter, or, as the author prefers, from Ohm's law directly. Since the cell itself is uniformly rectangular or cylindrical and the field is practically homogeneous throughout,

$$X = \frac{IR}{q},$$

where I = current; R = specific resistance of the suspension; q = cross-section of the cell.

Effect of Differences in Sign of Charge on Particle and Wall

In FIGURE 9 are shown types of curves obtainable in microscope experiments, when the net charge of the wall and of the particle are positive, negative, or zero.¹¹ Only if both particle and wall have a net charge of zero is no movement observed (curve 5). If both particle and wall are of the same sign, diminution in velocity always occurs at

¹⁵ Boswell, A. M., & Larson, T. E. *Jour. Phys. Chem.* 40: 833. 1936.

¹⁶ Henry, D. C. *Jour. Chem. Soc.* 997. 1938.

the wall and an increase occurs in the mid-regions of the cell. If the sign of charge differs, the opposite phenomenon occurs. The particles then have increased velocities near the wall due to the fact that the liquid streams near the wall of the cell in the same direction that the

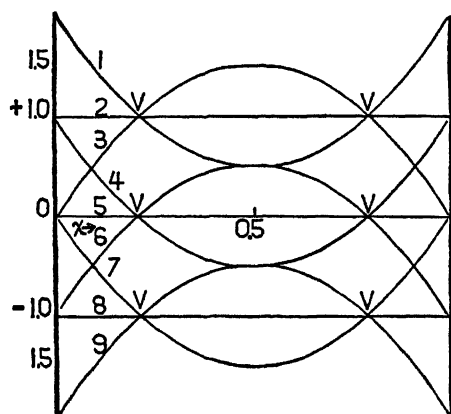


FIGURE 9. Nine types of curves obtainable in (flat cell) microscopic electrophoresis experiments.

particle moves. In the mid-regions of the cell, owing to the back-flow of liquid, the velocity of the particle is decreased. It is remarkable to see a system where the observed velocity is constant at all levels in the cell. This occurs when the net charge on the wall of the cell is zero, i. e., no electroosmosis occurs.

Rotating Field

A novel series of experiments in which a rotating electric field was devised so as to cause microscopically visible particles to move in circles has been described by Pugh and Swartz.¹⁷ The rotating field was produced by applying a two-phase alternating current source to four electrodes placed at the corners of a square in a thin electrophoresis cell constructed of a thin quartz chamber. The electrodes were formed by painting the proper design on the under side of the cover glass with gold paint and heating to 750° C. Over a small area in the center of the square, the corners of which are made up of the ends of the four gold electrodes, exists a uniform field constant in magnitude and rotating with constant angular velocity. Each particle then describes the circumference of a circle which will depend upon

¹⁷ Pugh, E. M., & Swartz, C. A. *Phys. Rev.* 36: 1495. 1930.

the mobility of the particle, the circumference being greater, the greater the electric mobility (FIGURES 10 and 11).

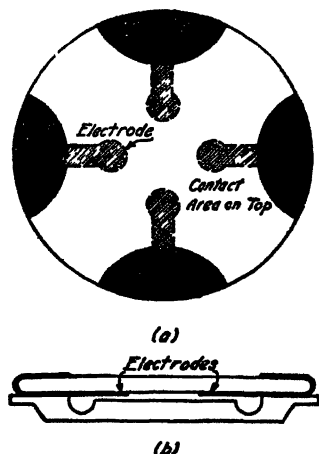


FIGURE 10. (a) Quartz cover glass with gold electrodes to produce rotating field. (b) Cross section of electrophoresis cell with cover glass. (Pugh and Swartz.)



FIGURE 11. The path of an oil droplet in a rotating field (Pugh and Swartz).

The diameter of the orbits described by oil droplets observed in the apparatus by Pugh and Swartz fluctuated considerably. These fluctuations were too rapid to enable Pugh and Swartz to make quantitative visual observations. The amount and rapidity of these fluctuations depended upon the size of the particles, similar to the way in which Brownian movement depends upon size. They were hardly perceptible with particles greater than 1μ in diameter and were independent of the chemical constitution of the particle. The writer has observed a phenomenon in all probability similar to the fluctuation phenomenon just described. Very small particles migrating in a direct field undergo deviations from a straight line in their path, which appears to be somewhat irregularly wave-like in nature. They have not as yet been studied in detail by photographic procedures.

The cell used by Pugh and Swartz was very thin, about 10μ . In a cell as thin as this and constructed as indicated, there is apparently no return flow of the liquid due to electroosmosis at the point where the particles are studied. The liquid in the small area under observation moves with the same velocity at all levels. In consequence, the velocity of the particle is constant throughout the thickness of the

cell. Nevertheless, the velocity of the particle relative to the liquid is,

$$V_{OBS} = V + V_W,$$

both V and V_W in this case being a constant. It would be of interest to test this relationship for systems where $V = V_W$. This can be done by covering both particle and wall of the cell with an adsorbed layer of protein, having previously determined the value of V .

Alternating Field

Cotton and Mouton¹⁸ have developed a method for the examination of the linear path described by a particle moving in an alternating field. Svedberg and Andersson and others have developed procedures to quantify these observations.

Rotating Cell of Cylindrical Cross-Section^{19,15,16}

McTaggart has described the apparatus depicted in FIGURE 12.



FIGURE 12. Rotating electrophoresis cell for the study of gas bubbles. (McTaggart)

The glass stopcocks are arranged to carry platinum electrodes, between which a small gas bubble is acted upon by the impressed electric field. By rotating the horizontal tube with sufficient velocity the gas bubble moves toward and remains in the central axis of the tube. The fact that the velocity at the center of the tube is independent of the tube's diameter does not signify that $V_{0.5}$ is the velocity of the bubble relative to the liquid as has been frequently supposed. Before data so obtained can be given quantitative weight the curve of velocity at different levels must be analyzed in terms of the pertinent equations for cylindrical cells.²⁰

Double-tube Cell

It would seem that the methods used by the writer and by Moyer have been shown by virtue of numerous experiments involving com-

¹⁸ Cotton, A., & Mouton, H. *Compt. rend.* 138: 1584, 1692. 1904.

Svedberg, T., & Andersson, H. *Kolloid. Z.* 24: 156. 1919.

Bluh, O. *Ann. physik*, 78: 177, 1925. 79: 143. 1926. 80: 181. 1926.

¹⁹ McTaggart, H. A. *Phil. Mag.* 27: 297. 1914. 28: 367. 1914. 44: 386. 1922.

Alty, T. *Proc. Roy. Soc. (London)* A106: 315. 1924.

²⁰ Bach, N., & Gilman, A. *Acta Physico-chimica* 9: 1. 1938.

Klemm, A. *Physik. Z.* 39: 783. 1938.

parisons of the same systems with different methods, to be the most satisfactory at present for measurements on biological systems heterogeneous in nature. However, the further development of a new technique presented by M. E. Smith and Lisse²¹ may increase the precision of the method. Smith and Lisse employ two tubes in parallel, T_1 and T_2 , in a closed system between platinum electrodes (FIGURE 13).

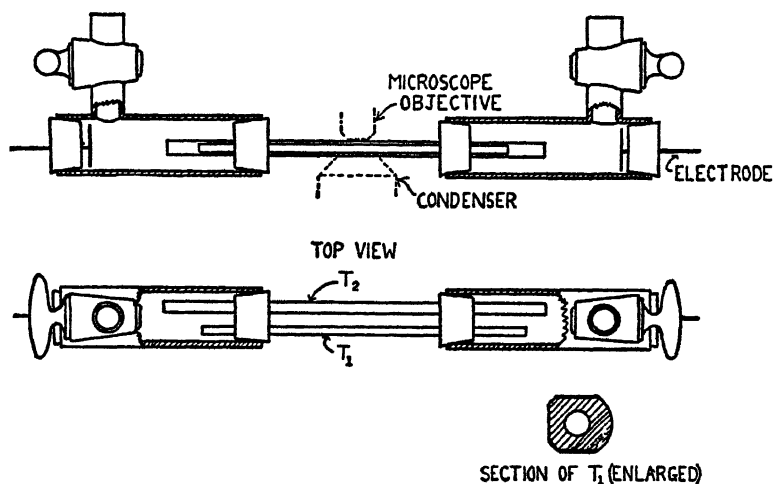


FIGURE 13. The double-tube cylindrical electrophoresis cell of Smith and Lisse.

The relationship between the tubes is so fixed that the electroosmotic return flow, ordinarily taking place in the mid-regions of the single tube microelectrophoresis cell, takes place in the tube T_2 of larger radius. Further, there is no movement of the liquid along the axis of the tube of smaller radius. Hence the velocity occurring at the center of T_1 (depth = $\frac{1}{2}$) corresponds exactly to the velocity of the particle relative to the liquid, for in this system the "stationary level" is at the center of the cell. The velocity gradient is zero at this level, a situation which theoretically is also more desirable. In the single cell of cylindrical cross section, the velocity relative to the liquid is at the level 0.146 (0.854) while there is only one stationary level in the double-tube cell.

Direct Application of Electrodes

An attempt to investigate the electrokinetic potential of the leaf cells of *Elodea* has been made by Rubinstein and Uspenskaja.²²

²¹ Smith, M. E., & Lisse, M. W. Jour. Phys. Chem. 40: 399. 1936.

²² Rubinstein, D. L., & Uspenskaja, V. Protoplasma 21: 191. 1934.

Leaves were placed on a slide in a plasmolysing solution in order to make the protoplasm round up within the box-like compartments of the cell wall. It was found that the protoplast moved to the positive ends of their cell compartments under the influence of an external field. Sen²² has carried this type of research even further, observing directly the motion of protoplasmic particles within the intact hairs of *Urtica dioica* (nettle) and root hairs of *Azolla pinnata* (an aquatic fern). These cells were put on a coverslip, surrounded with mineral oil and inverted as a hanging drop preparation (FIGURE 14). In this

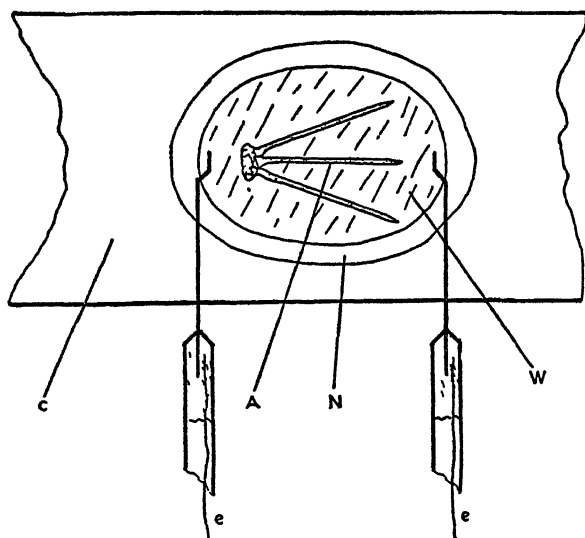


FIGURE 14 The arrangement of Sen for investigation of intracellular electrophoresis
N, Nujol, W, Water, A, Azolla cells, C Coverslip, e, Electrodes

position the particles in the cells continue in active Brownian movement for at least 24 hours. An electric field was applied by means of micro-electrodes of platinum (Pt-Ir, Ag-AgCl or agar gave the same results) held in a micromanipulator. These electrodes were not inserted into the cell but placed at both ends of the preparation. Upon applying the field to the *Urtica* hair, the intraprotoplasmic particles were seen to move toward the anode when observed under dark-field. If the field was reversed, reversal in direction occurred nearly instantaneously. A gradient of 4 to 40 volts was established between the electrodes, but there was no way of knowing just how much of this field strength was operating on the particles. With

²² Sen, B. Ann Bot 48: 143 1934

currents higher than 15 μ amp., and of a duration longer than 5 seconds, coagulation resulted; but under less severe conditions, the current could be applied and broken at least 12 times with no observable injury.

In *Azolla* the particles carried a net negative charge. It was found that the cylindrical hair apparently functions as a cylindrical electrophoresis chamber since particles moved with different mobilities at different levels in the cell. Sen reports that (1) particles near the cell wall scarcely moved, or, if at all, toward the cathode; (2) particles a slight distance away from the wall moved toward the anode; and (3) particles at the axis of the cell showed a maximum mobility irrespective of their sizes and shapes. On breaking the field, the particles at position (2) reversed in direction of motion for a short time. Although Sen does not point it out, such behavior of particles, if observed in an electrophoresis cell, would probably be interpreted as indicating a nearly identical surface constitution for the particle and the wall of the cell. Whether the surface causing the equal and opposite electro-osmosis at the wall (although the surface itself must be negative) is to be identified with the inner side of the plasma membrane, cannot, of course, be decided now, but Sen's results suggest possibilities of investigating it in this indirect way.

TABLE 1
THE RESULTS OF SEN FOR ELECTROPHORESIS IN A PLANT CELL

Approximate Diameter of Particle	Volts Applied	Distance in Time, t	Time, sec	μ /sec	μ /sec/volt
0.5	2	26	5	5.2	2.6
2.0	2R*	16	3	5.3	2.6
1.0	2	16	3	5.3	2.6
2.0	2R	22	4	5.5	2.7
0.5	4	32	3	10.6	2.6
2.0	4R	21	2	10.5	2.6

* R indicates reversal of current

TABLE 1 shows Sen's results with large and small particles in an *Azolla* hair 1.67 mm. in length and 22 μ in diameter. The velocities given are for particles at the axis. Since we have essentially a parallel circuit, the current being divided between the water and the hair, doubling the applied field would probably double the potential gradient in the hair. That this is true is shown in column 6 where the writer

and Moyer²⁴ have calculated the velocity in $\mu/\text{sec}/\text{volt}$. It will be noted that the constancy is quite good. This indicates the linearity of velocity with field strength, an observation which had not hitherto been reported for the electrophoresis of particles within living cells themselves.

THE CHARGE DENSITY

The Effect of Simple Salts on the Surface Density of Net Charge of Non-Ionogenic Surfaces

It has been shown in the first part of this paper that the effect of salts on v , the electric mobility of particles with non-ionogenic surfaces is a complicated one. Not only is there the usual decrease in v with increasing concentration of simple salts, but also peculiar maxima are observed in dilute solutions. (See FIGURE 2 for a typical ζ - c or v - c curve.)

How can this curious type of curve be explained? Theoretical considerations of this problem have indicated that the charging process of a surface must be considered. As we have emphasized before, the charge of the simple ion remains constant even though its mobility decreases. This is by no means the case when non-ionogenic surfaces are considered. As the concentration of salts increases, the net surface charge density changes as more ions are added to the solution. By means of equation 2, or one of its simplified forms, we have a means of following changes in the net surface charge with the concentration of the salt. It should be noted at this point that ionogenic surfaces like protein are not included in our present discussion. Data on the effects of salts on the charging process of protein are published elsewhere. Keeping in mind then the complex form of the ζ - c curve, examine FIGURES 15 to 19 which illustrate what occurs when the density of net charge σ is calculated.²⁵ Characteristic of the σ - c curve in every instance is, that with addition of salts, not reversing the sign of charge, σ increases sharply and almost linearly at low concentrations, with the charge density apparently reaching a limiting value at about $c = 0.001$ Molar. The descending part of the curve given in FIGURE 2 has frequently been taken to represent a discharge of the surface of a particle, since aggregation frequently occurs in this concentration range. FIGURES 15 to 19 demonstrate that, contrary to this notion, the addition of salt is actually accompanied by an increase

²⁴ Abramson, H. A., & Moyer, L. S. *Trans. Electrochem. Soc.* 71: 135. 1937.

²⁵ Abramson, H. A., & Mueller, H. *Bull. Proc. Am. Phys. Soc.* 7: 11. 1932. *Cold Spring Harbor Symposium* 1: 1933.

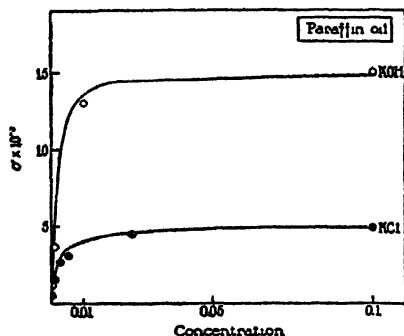


FIGURE 15. The points on the V - c curve have been calculated from data of Ellis published in 1911, and from data of Mooney (1931).

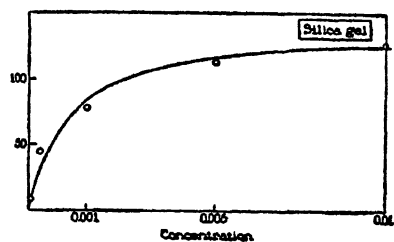


FIGURE 16. The effect of a simple salt on silica gel calculated from data of Glixell and Wiertelak. The smooth curves are typical isotherms.

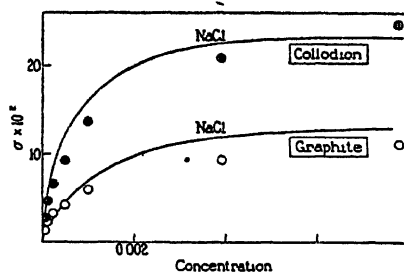


FIGURE 17. Calculated from data of Loeb.

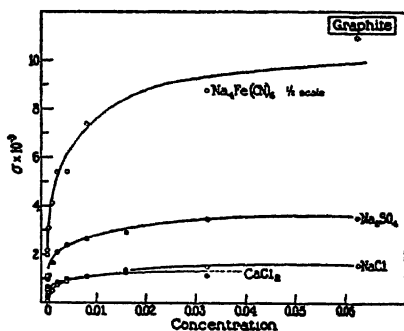


FIGURE 18. Calculated from data of Loeb.

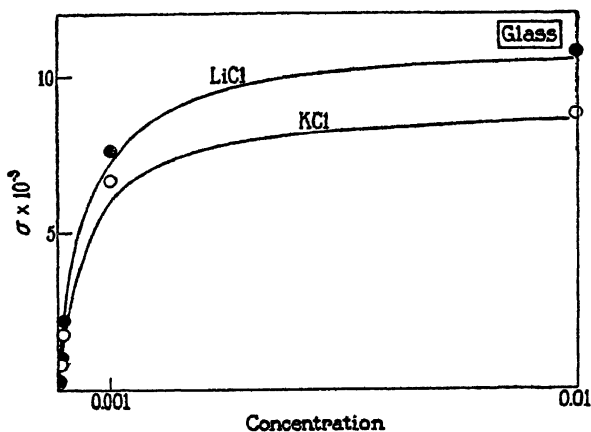


FIGURE 19. The effect of salts on the surface density of net charge on glass. From data of Furutani, Kurokuchi, and Asoda. The smooth curves have been drawn according to a form of the Langmuir isotherm.

of the net charge even in the region where it is known that rapid coagulation may occur. The course of the ζ - c or v - c curve is due to the fact that the mobility depends upon two variables: (1) the way in which the charge density of the surface changes; and (2) the way in which κ , the reciprocal thickness of the diffuse ionic atmosphere changes, with both of these being dependent upon c . ζ and κ presumably vary quite differently with c . As c increases $1/\kappa$ decreases; when c is small, the increase in σ is more important, that is, in low concentrations of salt the charging process by the ions is more important than the change in the diminution of the thickness of the double layer. But if c increases further, the decrease in $1/\kappa$ becomes more important in flattening out the curve as saturation occurs by what is probably ionic adsorption.

The reader will have noticed that the form of all the σ - c curves bears a strong resemblance to the simple adsorption isotherm. The smooth curves of FIGURES 15 to 19 have actually been plotted by an equation of the form,

$$\sigma = \sigma_M \frac{Bc}{1 + Bc}$$

where $\sigma_M B$ is the initial slope of the σ - c curve and σ_M is equal to the limiting value of σ within the concentration range. The agreement is excellent. It indicates that selective adsorption of the negative ion in all probability occurs. The curves for the different alkali halogens and alkaline earth halogen show fewer variations than the curves of potassium bromide, iodide and chloride. Especially large values of the net charge are obtained for the sulphates and ferrocyanides. The data indicate that for glass, graphite and collodion, the adsorption potential increases in the series Cl^- , Br^- , I^- ; Li^+ , Na^+ , K^+ , Rb^+ , Cs^+ ; Mg^{++} , Ba^{++} , Ca^{++} , and that in general, for these surfaces the adsorption potentials of the positive ions are considerably smaller than those of the negative ions.

It is of interest to calculate, neglecting the adsorption of positive ions, the fraction of the area occupied by the ions representing the net charge at a limiting value of σ . In the case of glass, σ is equal to 9,000 e.s.u., representing about 2×10^{13} ions per cm^2 . If the area occupied by each ion is taken as 5×10^{-16} cm^2 , the limiting area occupied by the excess negative charges is about 0.01 cm^2 or 1% of the surface. This is a rather higher value than that deduced by previous investigators, but is compatible with the simple type of adsorption postulated.

It may have been noted that the σ - c curves have been plotted so

that the curves pass through the origin. The σ - c curves represent not the net charge of the surface but the net charge due to the addition of ions.¹¹ This may be understood on the basis of the following reasoning. The values of σ given in the curves have been corrected for the original charge due to the ions in distilled water. In each case the total charge σ_T has first been calculated. This total charge is due both to the ions of the solvent and dissolved carbonic acid and to the ions of the added salt. If σ_W is due to the ions of the solvent and carbonic acid alone, the charge due to the salt is evidently,

$$\sigma = \sigma_T - \sigma_W$$

σ is, then, the value of the net charge due to the salt itself.

The σ - c curves of all other non-ionogenic surfaces which have been investigated in solutions of simple salts show the same characteristics found for those presented here.¹¹

The Effects of Salts on the Surface Density of Net Charge on Bacterial Surfaces

In TABLE 2 are calculation of σ from data of Northrop and DeKruif on the effect of salt on the charge density of unsensitized typhoid bacilli. As the concentration of salt increases the ζ -potential drops. However, σ , the charge density, increases from 224 to 924 E.S.U. Similar calculations (from the data of Mudd and Joffe) are presented in TABLE 3, where in addition to the drop in the ζ -potential, there is actually an increase in the charge density, with increasing salt concentration and agglutination, the charge density rising from 1420 E.S.U. to 3150 E.S.U. Further investigation of the effect of salts on ζ and on σ is desirable, especially of the simpler salts like KCl and Na_2SO_4 .

TABLE 2
INFLUENCE OF UNI-UNIVALENT SALT CONCENTRATION ON THE
CHARGE DENSITY OF UNSENSITIZED TYPHOID BACILLI

M	Agglutination	ζ volts	σ E.S.U.
0.0001	None	0.030	224
0.0005	None	0.025	417
0.001	None	0.022	530
0.01	None	0.010	700
0.05	None	0.006	924

TABLE 3

THE INFLUENCE OF THE SALT CONCENTRATION (NaCl) ON THE CHARGE DENSITY OF UNSENSITIZED "ROUGH" TYPHOID BACILLI

M	Agglutination	v $\mu/\text{sec.}$	ζ volts	σ E.S.U.
0.001	+	3.9	0.053	1,420
0.004	++	3.6	0.049	2,590
0.01	++	3.2	0.043	3,390
0.02	++	2.4	0.033	3,600
0.04	+++	1.6	0.022	3,150

More accurate data than those presented in the tables have been obtained by Moyer,²⁶ who studied the effect of acetate buffers of constant pH but varying ionic strength on the electric mobility and charge density of *E. Coli*. His curves are reproduced in FIGURE 20.

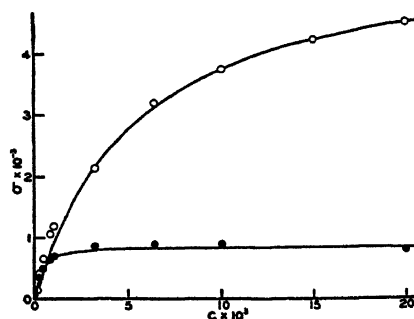


FIGURE 20. Charge density concentration curves of *E. Coli* in acetate buffers. Open circles; rough form. Closed circles; smooth form. (Moyer.)

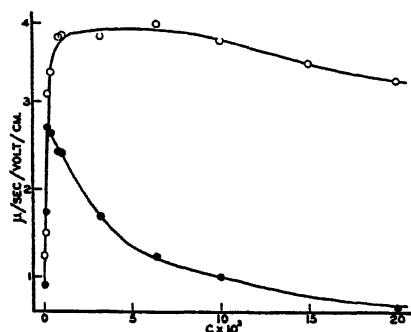


FIGURE 21. Mobility-concentration curves of *E. Coli* in acetate buffers. Open circles; rough form. Closed circles; smooth form. (Moyer.)

²⁶ Moyer, L. S. Jour. Bact. 32: 433. 1936.

Note that the smooth form (closed circles) reaches the maximum value of σ at approximately 0.001 M., whereas the rough form (open circles) is still increasing the value of its surface charge. Compare the σ - c curves with the v - c curves reproduced from Moyer in FIGURE 21. The remarkable constancy of σ_M in the lower curve of FIGURE 20 is both confirmatory of the general validity of the theory and a tribute to Moyer's experimental skill.

The Charge Density of Mammalian Red Blood Cells

Red blood cells may be readily studied by the microscopic method of electrophoresis.²⁷ The data given in the table show the wide difference in electric mobility of the blood cells in different mammals. Cells of young monkeys, young rabbits, have the same speed as those of adults. The following series shows the order found:

Rabbit	<	Sloth	<	Pig	<	Opossum	<	Guinea pig	<	Man
(0.55)		(0.97)		(0.98)		(1.07)		(1.11)		(1.31)
Monkey	<	Mouse,		Cat	<	Rat	<	Dog		
(1.33)		(1.40)		(1.39)		(1.45)		(1.65)		

The electric mobilities of the cells (given in parenthesis) are proportional to the ζ -potential. They do not give us any indication of the charge density or the net charge of each cell. Values of σ were calculated (TABLE 4), by means of equation (2) from the data ob-

TABLE 4

Animal	Mobil- ity	ζ	σ	Area	Net charge	Number of elec- trons	Area occu- pied
	μ /sec	volts	E.S.U.	$cm^2 \times 10^6$	E.S.U. $\times 10^3$	$\times 10^{-6}$	per cent
Rabbit	0.55	0.00704	1890	1.10	2.08	4.37	0.40
Sloth	0.97	0.0124	3330	—	—	—	—
Pig	0.98	0.0125	3360	0.95	3.19	6.70	0.70
Opossum	1.07	0.0137	3680	1.56	5.74	12.0	0.77
Guinea pig	1.11	0.0142	3780	1.15	4.35	9.14	0.80
Man	1.31	0.0168	4500	1.63	7.34	15.4	0.94
Rhesus monkey	1.33	0.0170	4570	1.37	6.26	13.2	0.96
Cat	1.39	0.0178	4780	0.80	3.82	8.03	1.00
Mouse	1.40	0.0179	4800	0.96	4.61	9.70	1.01
Rat	1.45	0.0186	4980	1.02	5.08	10.7	1.05
Dog	1.65	0.0211	5660	1.22	6.90	14.5	1.19

²⁷ Abramson, H. A. Jour. Gen. Physiol. 12: 711. 1929.

tained with various types of mammalian red cells²⁸ in isotonic phosphate buffer at pH 7.4. Through the kindness of Dr. E. Ponder the surface areas of these red cells were determined and, using these values, the net charge per cell was calculated from σ . It may be noted in TABLE 4 that the net charge of the red cell does not vary in the same order from species to species, as does the value of σ , the charge per unit area. Nor does there seem to be any clear relationship between the net charge per cell and zoological classification. These differences in mobilities may be directly observed in mixtures composed of the cells of different animals.

By dividing the net charge by the electronic charge the number of effective electrons at the surface has been calculated (TABLE 4, last column). For example, in the case of man there are 15,000,000 effective electrons on each cell, the highest value amongst these mammals. One might say this corresponds to the valence of each cell. A similar computation of the net charge has been made for the typhoid bacillus.¹¹ The values never rise far above 1%, which agrees in magnitude with the data obtained on the surfaces discussed in the foregoing and even with the ionic saturation values of protein molecules.

Ion Antagonism and the σ - c Curve

The ζ -potential of cellulose was found by Bull and Gortner²⁹ to show no evidence of ion antagonism when chlorides of various cations

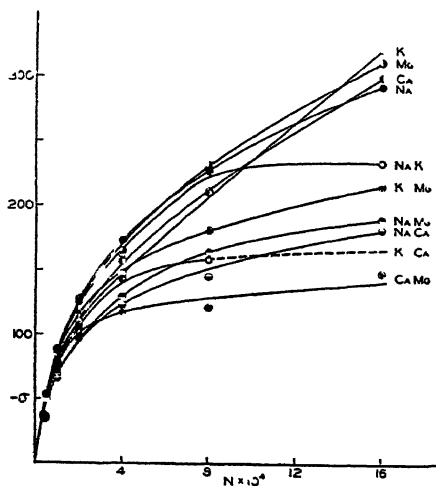


FIGURE 22. Charge density and ion antagonism. (Moyer and Bull.)

²⁸ Abramson, H. A., & Moyer, L. S. Jour. Gen. Physiol. 19: 601. 1936.

²⁹ Bull, H. B., & Gortner, E. H. Jour. Phys. Chem. 35: 700. 1931

were studied. However, by calculating the charge density as indicated in FIGURE 22, Moyer and Bull showed that definite ion antagonism exists at the surface. This is the first instance in which quantitative data have shown a connection between ion antagonism and electrical effects, although similar phenomena have been observed in coagulation studies.

The charge density of proteins may be analyzed by similar methods. However, ionogenic surfaces, the net charge of which are profoundly influenced by the pH of the medium, require special consideration. At present the data required for an analysis of these systems should include the effects of salts on the electric mobilities of proteins, the isoelectric points of which are not shifted appreciably by the salt.

The net charge of an ideal protein ion of this type should be determined primarily by the pH of the medium, and although its mobility would decrease without passing through a maximum (FIGURE 2) with the addition of salt, the net charge would remain constant as for a simple ion like potassium.

THE MOVING BOUNDARY METHOD FOR STUDYING ELECTROPHORESIS

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INTRODUCTION

The first electrophoretic experiment on record was performed by the Russian physicist Reuss in the year 1807. His apparatus consisted of two pieces of glass tubing which were driven into a block of wet clay and filled with water. A layer of sand was put on the bottom of the tubes which were then connected with the poles of a voltaic pile made of 74 silver-zinc couples. Upon closing the circuit the water in the tube connected with the positive pole assumed a milky appearance due to the electrophoretic migration of colloidal clay particles through the sand layer. At the negative pole the water remained clear but increased in volume thus exhibiting the phenomenon of electroosmosis. The observations of Reuss were confirmed and extended, among others, by Faraday and by Du Bois-Reymond. The first quantitative studies were undertaken by Wiedemann in 1856 and by Quincke in 1861. The measurements of the latter showed that the rate of migration of particles in a field is a linear function of the potential gradient.

The first workers to experiment with a V-shaped cell, the forerunner of the U-tube, were probably Picton and Linder (1892-1897). They studied the movement of boundaries of As_2S_3 , shellac, ferric hydrate, hemoglobin and other colloidal sols in dependence of the direction of the current and of the acidic or basic character of the colloid. By reversal of the direction of current they demonstrated the reversibility of the phenomenon.

Hardy, in his classical study on the electrophoresis of proteins in 1905, laid the foundations for much of the current work. With the aid of a U-shaped tube containing a layer of the colloid, *e. g.* of an opalescent globulin solution, under a supernatant fluid of the same electrolyte content he was able to perform quantitative determinations of the mobility of the boundary of the colloid.

The moving boundary method of studying the electrophoresis of colloids and in particular of proteins which form the object of most of the recent investigations owes much to the pioneering research by Lodge, Whetham, Nernst, Masson, Denison and Steele, MacInnes,

Kohlrausch, and other workers on the behavior of the ions of ordinary electrolytes in electric fields. For a discussion of the bearing of that work on the problem of electrophoresis of proteins and for a review of the earlier researches on the electrophoresis of proteins themselves reference is made to the book by Pauli and Valkó¹ and to the dissertation by Tiselius.² The technical development of the subject up to 1931 has been reviewed by Prausnitz and Reitstötter.³

SIMPLE U-TUBE ELECTROPHORESIS CELLS

In order to study electrophoresis by the moving boundary method it is necessary to stratify the solution of the substance under investigation, *e. g.* of a protein or polysaccharide, below a layer of the solvent, *e. g.* a buffer solution. Three methods are available for the formation of the well-defined boundary required for this purpose, viz. (1) establishment of contact between solution and solvent with the aid of stopcocks after attainment of temperature equilibrium, (2) controlled flow of the solution containing the colloid (under hydrostatic or air pressure) below the solvent causing a gradual displacement of the latter, and (3) mechanical withdrawal of slides separating the solution from the solvent or the equivalent mechanical alignment of sections filled with solution and solvent respectively. Arrangements employing these various principles will be described below.

The first U-tube cell with stopcocks in each limb for the formation of the boundaries was described in 1908 by Landsteiner and Pauli. A modification of this cell, employing as an important improvement reversible electrodes, has been extensively used by Michaelis and his students.⁴ It is shown in FIGURE 1.

The direction of migration of the colloid may either be observed visually, provided the substance is colored or opalescent, or it may be ascertained by closing the stopcocks A and B at the end of the experiment and subsequent analysis of the fluids in compartments 2 and 4 for the presence of the colloid. If the substance is endowed with catalytic properties, *e. g.* enzymatic activity, the sense of migration at a given hydrogen ion concentration may be determined even in quite crude or highly diluted preparations with the aid of suitable catalytic test reactions. This simple cell has proved its usefulness for

¹ Pauli, W., & Valkó, L. *Electrochemie der Kolloide*. Dresden & Leipzig 1933.

² Tiselius, A. *Nova Acta Soc. Sci. Upsala*. IV 7 (4). 1930.

³ Prausnitz, P. H., & Reitstötter, J. *Electrophorese, Electroosmose, Electrodialyse in Flüssigkeiten*. Dresden & Leipzig 1931.

⁴ Michaelis, L., & Bona, P. *Praktikum der Physikalischen Chemie*. Julius Springer, Berlin 1930.

the determination of the isoelectric point of various biocolloids such as hemoglobin, invertase and catalase.⁵

While this apparatus is suitable as a "null instrument," *i. e.* for establishing the pH at which the mobility of the colloid becomes zero or at which the direction of migration will change, it cannot be used for absolute mobility determinations on account of the inhomogeneity of the field caused by the various restrictions in the path of the current.

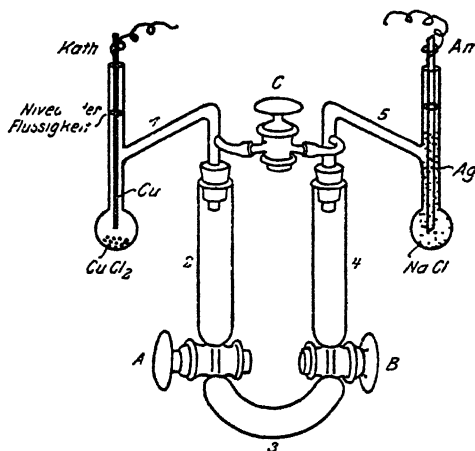


FIGURE 1. Landsteiner and Pauli's Electrophoresis Cell, as modified by L. Michaelis. The sol to be studied is placed in compartment 3, while 2 and 4 are filled with the buffer solution. The side tubes 1 and 5 are also filled with buffer solution and serve as connections to the electrode vessels. The nature of the electrodes is indicated in the figure. The stopcock C is opened briefly to equalize the hydrostatic pressure in the cell and is kept closed during the electrophoresis. Contact between the sol and the supernatant buffer is made by cautiously opening the stopcocks A and B. In order to avoid boundary disturbances due to the restriction of the tube by the stopcocks, the density of the sol in compartment 3 is increased by adding some sucrose to it. For supply of current the apparatus may be connected with the electrical mains carrying 110 or 220 v. D.C.

In a second apparatus, designed by Michaelis⁶ for rate measurements, the cross-section of the U-tube is maintained uniform throughout by the use of wide-bore stopcocks. The limbs of the tube above the stopcocks are graduated so as to permit readings of the position of the boundaries free from parallax (FIGURE 2).

In order to avoid thermal convection currents which tend to destroy the definition of the boundary the cell must be immersed in a water thermostat and the heat produced by the current must be kept as small as possible (see below). The cell has been used for measurements

⁵ Michaelis, L., & coworkers. *Biochem. Z.*, 16: 81, 486. 1909. 19: 181. 1909. 29: 439. 1910. 33: 456. 1911. 41: 102, 373. 1912. 47: 260. 1912. 53: 320. 1913.

⁶ Michaelis, L., & Airila, Y. *Biochem. Z.*, 118: 144. 1921.

of the electrophoretic mobility of a number of colloids, *e. g.* mastix sols, hemoglobin,⁶ etc., and for the determination of the isoelectric point of various enzymes, *e. g.* catalase,⁷ and biological pigments, *e. g.* ovoverdin, the carotenoid-protein of the egg of the lobster.⁸

In its original form the electrophoresis cell of Michaelis yields only three fractions for subsequent analysis, *viz.* the fluid in the lower

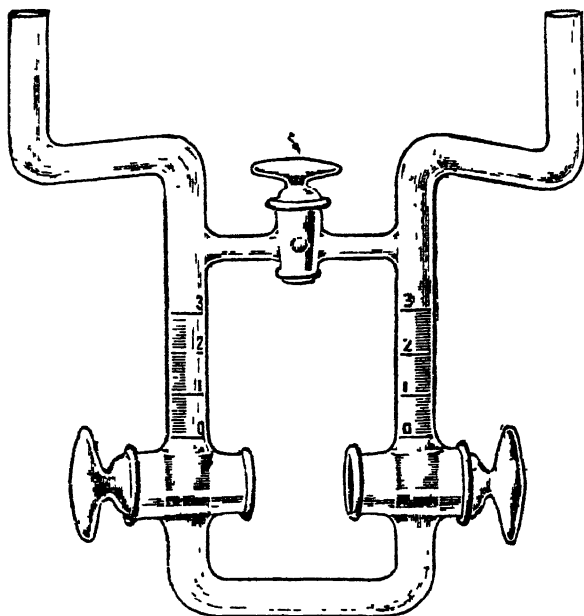


FIGURE 2. Electrophoresis Cell for Quantitative Experiments acc. to Michaelis. Contact with the electrode vessels is established by agar-KCl bridges. Both electrodes are of copper and dip into a CuSO_4 solution. Since the mechanism of depolarization is symmetrical here, the direction of the current may be reversed during an experiment in contrast to the arrangement shown in FIGURE 1. A suitable potential gradient is about 3 volt/cm. It is produced by applying an external current of 100 volt to an U-tube of 32 cm. length (as measured between the tips of the agar bridges which are not shown in the figure).

part of the U-tube under the two stopcocks and the solutions obtainable from the side limbs above the stopcocks. The provision of two additional openings at the top of the side limbs as suggested by H. Theorell makes it possible to obtain a much greater number of samples. A large series of experiments on blood sera and serum proteins has been performed by Bennhold⁹ with the aid of such a modified cell.

⁵ Stern, K. G. Z. Physiol. Chem. 208: 86. 1932.

⁶ Stern, K. G., & Salomon, K. Jour. Biol. Chem. 122: 461. 1938.

⁹ Bennhold, H. Ergebn. inner. Med. & Kinderheilkunde 42: 273. 1932. Kolloid. Z. 62: 129. 1933.

Bennhold was able to show that the bilirubin present in serum is quantitatively linked to the albumin component whereas the cholesterol migrates with the globulin fraction. When naphthol yellow and sudan red are added in small amounts, the former will migrate with the albumin and the latter with the globulins thus allowing direct visual observations of the electrophoretic separation of the serum proteins. If the dyes are added in excess the portion not bound by the protein exhibits the high mobility of low-molecular ionized particles (see FIGURE 3). As another example for the usefulness of this simple apparatus the following experiment may be quoted. Chemically purified liver catalase solutions exhibit maxima of light absorption in the visible range of the spectrum at 622 and at 409 $m\mu$. The disappearance of the band at 622 $m\mu$ upon adding ethyl hydrogen peroxide and its gradual reappearance accompanying the breakdown of this substrate (Stern) as well as other observations showed that the enzyme itself was responsible for the absorption maximum in the red. In order to determine whether the maximum situated in the violet (at 409 $m\mu$) is also due to the enzyme or to an impurity a liver catalase preparation was subjected to electrophoresis in the modified Michaelis cell. The pigment layer was permitted to migrate in a field of 0.95 v cm at 24° for 26 hours (specific conductivity, 0.0091). At the hydrogen ion concentration chosen (pH 6.78) the enzyme molecule carries a negative charge and is sufficiently stable under the conditions of the experiment. Three fractions were obtained by pipetting off three layers, each 2 cm. high, of the pigmented solution contained in the anode limb of the apparatus, and the relative absorption spectrum of each fraction was recorded with the aid of Hardy's automatic photoelectric spectrophotometer. The substantial agreement of the ratios of the densities measured at 409 and 622 $m\mu$, *viz.* 10.3, 11.4 and 11.5, supports the view that both absorption maxima are caused by the same substance, *i. e.* by the enzyme catalase.¹⁰

QUANTITATIVE ELECTROPHORETIC STUDIES BY THE LIGHT ABSORPTION METHOD

The theory, technique and application to the electrochemical study of proteins of the light absorption method are treated in detail in the classical publication by Arne Tiselius.² This work had grown out of preliminary experiments by Svedberg with Jette¹¹ and Scott¹² who

¹⁰ Stern, K. G. *Biochem. Jour.* **121**: 561. 1937.

¹¹ Svedberg, T., & Jette, E. B. *Jour. Am. Chem. Soc.* **45**: 954. 1923.

¹² Scott, N. D., & Svedberg, T. *Jour. Am. Chem. Soc.* **46**: 2700. 1924.

took photographs of egg albumin solutions during electrophoresis utilizing the green fluorescence emitted by the protein when irradiated with long-wave ultraviolet light. The long time of exposure required (10 min.), the impossibility to immerse the apparatus in a water thermostat, and the risk of contamination by other fluorescent substances made the method seem impractical. Consequently Svedberg and Tiselius¹³ sought to improve the method of optical observation of electrophoresis by using the fact that all proteins will absorb more or less strongly radiation of wave lengths below 300 m μ . The absorption maximum of proteins is usually situated close to 270 m μ . In these preliminary experiments, performed with egg albumin near the isoelectric point, the rate of electrophoretic migration was determined by direct measurement of the distances on the photographic plate, a procedure yielding only approximate values due to the lack of definition of the boundaries and the small distances involved. The principle of following the migration of protein boundaries with the aid of short-wave ultraviolet radiation was first applied successfully by Svedberg and his collaborators to the study of the sedimentation of proteins in the ultracentrifuge.

The general arrangement of the apparatus employed by Tiselius is indicated in FIGURE 4. The choice of long focal distances of the

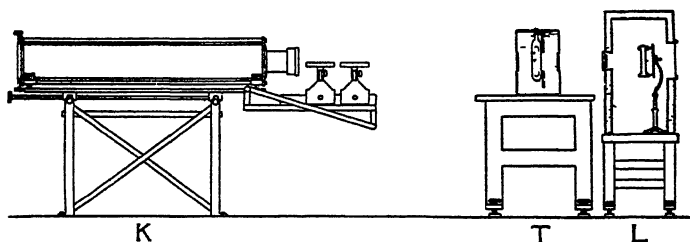


Fig. 4

FIGURE 4 Arrangement of Electrophoresis Apparatus of Tiselius for Light Absorption Method. L, Lamphousing, double-walled and water cooled, containing as the light source a quartz mercury lamp of the vertical type (Heraeus), run on 120-130 v from storage batteries. In front of the housing is a screen of ground, fused quartz. T, Water thermostat, maintained at 20° within 0.01°, stirred by a detached motor stirrer and equipped with plane parallel windows of bubble-free fused quartz. The tank contains the electrophoresis cell (see FIGURE 5) in a mount insuring an exactly defined position of the cell and at the same time serving as a diaphragm to screen out all radiation except that passing through two rectangular openings behind the limbs of the U-tube. K, Camera, equipped with a biconvex quartz lens of 630 mm focal length at $\lambda = 253 \text{ m}\mu$ and a combination of chlorine and bromine filters transmitting only radiation below 280 m μ to which the plates used (Imperial Process Plates) are sensitive and visible light in the yellow and of longer wave length to which the plates employed are insensitive. The camera is equipped with a movable plate holder accommodating 20 exposures (7.5 \times 35 mm) on one 9 \times 12 cm plate. Magnification ratio about unity.

camera lenses is necessary to avoid parallax. Mechanical vibrations are excluded by suitable shock-proofing.

The electrophoresis cell used by Tiselius is shown in FIGURE 5.

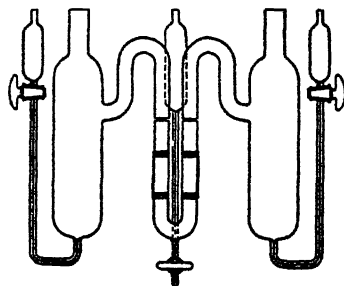


FIGURE 5. Electrophoresis Cell according to Tiselius.

The portion of the U-tube in which the migration of the boundaries of the protein solution is recorded by ultraviolet photography consists of fused bubble-free quartz. The two cylindrical limbs are ground with precision to yield a uniform cross section (0.76 cm^2). The migration of the boundaries is followed within an area enclosed by two indices (30 mm distance). The bottom of the U-tube is connected through a capillary and a stopcock with the storage vessel for the protein solution. This vessel as well as the remainder of the cell are made of glass and connection is made with the quartz U-tube by rubber tubing. The comparatively large electrode vessels are connected with KCl storage containers through glass capillaries. The electrodes are of the silver-silver chloride type and are immersed in saturated KCl solution. The current needed is supplied by a storage battery of 50 cells (100 v).

The experimental procedure is briefly as follows. The electrode vessels and the U-tube are filled with the supernatant buffer to be used in the experiment. The buffer must have the same pH and the same conductivity as the protein solution. This condition can be met by dialyzing the protein solution against the supernatant buffer or by the addition of a neutral salt, *e. g.* KCl, to the solution of lower conductivity under conductometric control. While the stopcocks are kept closed the central storage vessel in the back of the cell is filled with the protein solution and the two side storage vessels are filled with saturated KCl solution. After placing the electrodes into the large cylindrical vessels the cell is immersed in the water thermostat. When temperature equilibrium is reached the two side stopcocks are opened and the KCl solution is allowed to flow slowly into the elec-

trode vessels and to stratify there under the buffer solution. The electrodes are now surrounded by concentrated KCl solution as is required for reversibility of the electrodes. Now the stopcock connecting the U-tube with the protein container is cautiously turned until a flow of the protein solution through the capillary tube is just perceptible. Tiselius recommends to increase the pressure gradually by air pressure regulated by a water manometer arrangement. Experience in this laboratory has shown that satisfactory boundary formation may also be obtained by refilling the protein container up to the original level from time to time and thus permitting the protein solution to enter the U-tube under its own hydrostatic pressure. The formation of a sharp boundary is an essential condition in this method and it requires a little experience. In the light absorption method the protein concentration is limited to a narrow range if the photographic image is to show the correct contrast. On the other hand, the higher the density of the protein solution the easier is the stratification and the smaller becomes the risk of boundary disturbances due to thermal convection during electrophoresis. The protein concentration satisfactory for the diameter of the quartz tube employed by Tiselius (9.9 mm) was found to be 0.1 to 0.4 per cent. When the protein boundary has reached the middle between the two indices on the U-tube the stopcock is closed and the current is switched on. Photographic exposures are taken in regular intervals, depending on the mobility of the protein at the hydrogen ion concentration chosen. After some time the direction of the current is reversed and more exposures are taken while the boundaries move back to the original position. A series of pictures secured in this way is reproduced in *FIGURE 6*.

After the completion of a run the U-tube is filled with a series of different concentrations of the protein used in the experiment and photographs are taken on the same plate in order to obtain a concentration scale giving the relation between the actual protein concentration and the degree of blackening of the photographic plate. The plate is then analyzed with the aid of a registering microphotometer. The curves thus obtained form not only the base for the calculation of the electrophoretic mobility of the protein but their shape indicates also the degree of homogeneity of the preparation examined. If the solution contains only one component of a high degree of electrochemical homogeneity the slope of the photometer curves remains essentially unchanged throughout the course of the experiment, the only change which takes place being the distance of the boundary from

the indices. A minor degree of homogeneity is indicated by a change in slope of the photometer curves with time, *i. e.* by a spreading of the protein boundary in excess of that normally occurring due to diffusion. The presence of several electrochemically different components is revealed by distinct steps in the photometer tracing. The mobility, u , is defined as the distance of migration per second per volt, expressed in cm. For proteins it is found of the order of 10^{-5} cm² v⁻¹ sec⁻¹. A protein is perhaps best characterized in electrochemical respect by the value of the slope of the pH-mobility curve near the isoelectric point ($du/d \text{ pH}_0$). But it is often desirable to construct, from a

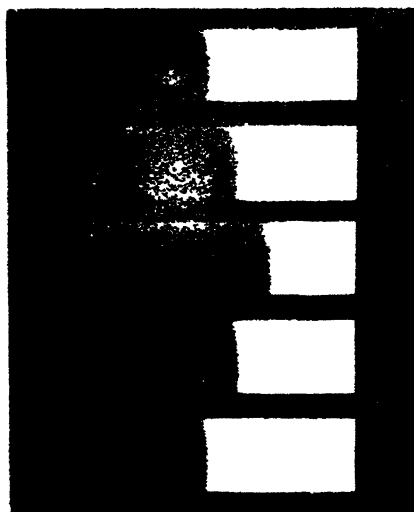


FIGURE 6. Electrophoresis of C-phycoerythrin, a chromoprotein from algae, with the light absorption method (Tiselius).

number of experiments at varying hydrogen concentrations, a complete pH-mobility curve.

The accurate determination of the mobility requires an accurate knowledge of the potential gradient existing in the U-tube during the experiment. Contrary to the usage of reading off the voltage on the apparatus and dividing it by the distance between the electrodes Tiselius prefers to measure the current intensity at frequent intervals and to obtain the potential gradient, F , with the aid of the formula

$$F = \frac{i}{q \kappa},$$

where i = current intensity in terms of ampere, κ = conductivity of

the solutions, and q = cross section of the U-tube at the place where migration of the boundaries occurs.

It is of importance to ascertain the extent of heat production in the apparatus during electrophoresis. The heat w watt produced per cm^3 and second in a tube of the cross section area q cm^2 at a current intensity i ampere and a conductivity κ is obtained by

$$w = \frac{i^2}{q^2} F^2 \kappa.$$

The heat production is therefore independent of the dimensions of the U-tube. In test experiments with alternating current Tiselius found that with a protein concentration of 0.2 per cent heat effects may cause boundary disturbances if the heat production exceeds 10^{-2} watt per cm^3 . Marked thermal disturbances manifest themselves by sharpening of the boundary and by the upward streaming of a fine column of dilute protein solution in the center of the boundary (see also Tiselius¹⁴).

For a discussion of other boundary anomalies reference is made to Tiselius' paper.²

The following well-defined proteins were studied by Tiselius with the aid of the light absorption method: Egg albumin, serum albumin, the algae chromoproteins phycoerythrin and phycocyan, Bence Jones protein, helix hemocyanin. All of these proteins are sufficiently soluble in the vicinity of the isoelectric point to permit a determination of the value of $du/d \text{pH}_0$. It was found that the position of the isoelectric point depends to some extent upon the composition of the medium. In the case of egg albumin there exists a distinct discrepancy between the isoelectric point and the point of minimum acid-base binding capacity. From the mobilities in the isoelectric region and the molar frictional coefficients as determined with the ultracentrifuge the apparent charge of the proteins could be calculated. Experiments with artificial protein mixtures, *e. g.* Bence Jones protein plus phycoerythrin or egg albumin plus Bence Jones protein, and with heterogeneous natural protein preparations, *e. g.* serum globulin, demonstrated the value of the method as a test for homogeneity. It was possible to study the effect of purification of protein preparations on the electrophoretic diagram. Thus an egg-white preparation from which the globulin component had been removed by dialysis yielded a distinctly distorted curve upon photometry of the migrating boundary indicative of a substance moving in the same direction as egg

¹⁴ Tiselius, A. Trans. Faraday Soc. 33: 524. 1937.

albumin but with a greater mobility (probably ovomucoid). On the other hand it was found that a material which proves to be non-uniform in the analytical ultracentrifuge does not necessarily exhibit inhomogeneity in the electrophoresis apparatus. A case in point is the phenomenon of reversible pH-dissociation of large protein molecules at hydrogen ion concentrations far from the isoelectric point. C-phycocyan and phycoerythrin, for example, which according to Svedberg and Katsurai tend to dissociate into smaller particles at pH 6.8 and 11 respectively are electrochemically homogeneous under the same conditions. This fact demonstrates clearly that the rate of migration in an electrical field is primarily a function of the *charge* on the protein molecule and is only little influenced by its *shape* or *size*. As long as the charge remains constant the mere dissociation of macromolecules into smaller aggregates does not manifest itself upon electrophoretic analysis. It is to be expected that factors of secondary importance with regard to electrophoretic mobility, *e. g.* shape and size, will be more readily detected in experiments at high mobilities, *i. e.* when working far away from the isoelectric point, whereas information concerning the uniformity of the particles with regard to the isoelectric point may best be gained when operating at low mobilities. A so-called "isoelectric zone" where migration takes place towards both electrodes at the same time is always indicative of non-homogeneity of a protein preparation. A single well-defined protein should show an equally well-defined point of reversal of direction of migration upon varying the hydrogen ion concentration.

Following the original work of Tiselius with the light absorption method the apparatus has been used by Tiselius and other investigators in Svedberg's laboratory in a number of investigations. As far as the reviewer is aware this type of arrangement has not been duplicated elsewhere, probably owing to the difficulties and expenses involved in obtaining the necessary quartz parts of high optical quality. Up to this date, however, the method appears to be capable of yielding the most precise values obtainable for electrophoretic mobilities and therefore also for the position of the isoelectric point. As a test for electro-chemical homogeneity, however, it has been somewhat supplanted by the introduction of the Toepler schlieren method as the procedure of optical analysis (see below).

The light absorption method, employing both short-wave and long-wave ultraviolet light, has been applied to the electrophoretic study of a number of respiratory pigments, *e. g.* various hemocyanins and erythrocrucorins, and in particular to the determination of their iso-

electric points and their $du/d\text{pH}_0$ -values, by Pedersen¹⁵ and by Svedberg et al.¹⁶ The measurements showed that the isoelectric point of respiratory proteins varies from species to species while the molecular weight remains frequently the same. The isoelectric point of the hemocyanin from *Helix pomatia*, for example, is 5.05 while that from *Helix nemoralis* is 4.63, both proteins having a molecular weight of 6,400,000 near the isoelectric point. On the other hand it was found, in confirmation of the earlier work of Tiselius on the algae proteins² that at pH 8.2 the hemocyanin of *Helix pomatia* has three well-defined components of molecular weight 6,400,000, 3,200,000 and 800,000, all of which are identical in electrophoretic behavior.

In the course of their experiments on crystalline preparations of Stanley's tobacco mosaic virus protein Eriksson-Quensel and Svedberg¹⁷ detected a certain degree of molecular inhomogeneity in virus protein preparations which had been repeatedly recrystallized. In spite of this lack of homogeneity in the ultracentrifugal field the samples showed a very uniform electrophoretic migration. The isoelectric point was found to be 3.49 and the slope of the mobility pH-curve ($du/d\text{pH}_0$) 12.3×10^{-5} . Later work by Stanley and Wyckoff has shown that the polydispersity of these early samples of the virus protein had been induced by the chemical treatment involved in their preparation (exposure to high salt concentrations) whereas virus protein preparations obtained by quantity ultracentrifugation are monodisperse.

Tiselius,¹⁸ in 1937, reinvestigated the problem of electrophoresis of the normal serum globulin fraction of the horse and the rabbit, and while he was able to establish the general shape of the pH-mobility curve of the preparations he concluded that both protein preparations are electrochemically heterogeneous. The results obtained in this study were to be somewhat revised and amplified a little later with the aid of the new apparatus employing the Toepler schlieren method (see below). A study of antibody preparations isolated by Heidelberger from type specific antipneumococcus horse and rabbit sera, conducted at the same time (Tiselius¹⁹) indicated that the pH-mobility curves of these preparations are distinctly different from those of normal serum proteins. Such a difference in electrophoretic properties, in turn, is strongly suggestive of differences in chemical constitution between the proteins studied.

¹⁵ Pedersen, K. O. *Kolloid. Z.* **63**: 268. 1933.

¹⁶ Svedberg, T., et al. *Jour. Biol. Chem.* **103**: 311. 1933.

¹⁷ Eriksson-Quensel, I. B., & Svedberg, T. *Jour. Am. Chem. Soc.* **58**: 1863. 1936.

¹⁸ Tiselius, A. *Biochem. Jour.* **31**: 313. 1937.

¹⁹ Tiselius, A. *Jour. Exp. Med.* **65**: 641. 1937.

APPARATUS FOR ANALYTICAL AND FOR PREPARATIVE PURPOSES OF H. THEORELL

If we except the application of electrophoretic methods to industrial problems (cf. Prausnitz and Reitstoetter³), it may be stated that up to 1934 electrophoretic experiments in biochemical research laboratories had been mainly conducted either with a view of determining by optical methods the electrical mobility and the isoelectric point of colloids of the type of proteins *or* in order to ascertain the sense of migration under a given set of conditions and, if possible, to effect a resolution of multi-component systems. H. Theorell developed equipment which cannot only be applied to studies of that type but which furthermore may be used for the electrophoretic purification and isolation of biocolloids.

Apparatus for Analytical Studies

In designing this small-scale electrophoresis cell Theorell²⁰ attempted to satisfy the following conditions: (1) The cross section of the U-tube must be sufficiently large to permit the performance of analyses of the contents and activity of several successive layers of relatively small height which should be located both below and above the original level of the boundary between the colloid and the buffer solution; (2) The U-tube should be of uniform diameter throughout to allow the calculation of the potential gradient for any part of the tube and thus the computation of the electrophoretic mobility; (3) Connection between the successive layers in the U-tube and mechanical separation should be accomplished without the risk of disturbing boundaries or concentration gradients; (4) The wall thickness of the U-tube should be uniform to prevent thermal convection currents set up by uneven heat distribution during the run and due to differences in thermal insulation and conduction; (5) The sol of the colloid under investigation must be sufficiently far removed from the electrodes to protect it from the diffusion of products of electrolysis. The arrangement designed to meet these requirements is depicted in FIGURE 7.

A view of the complete apparatus is shown in the left half of the figure. The U-tube (16 mm diameter) is built up of 12 cylindrical glass cells, each 10 mm high, planed off and polished, the form of which is indicated in the right lower half of the figure. There are interposed between these cells hard rubber disks, turning around a central axis, 0.25 mm thick in the region adjoining the glass cells, as

²⁰ Theorell, H. *Biochem. Z.* 275: 1. 1934.

shown in the right upper half of the figure. By manipulating the lever *d*, contact between two adjoining glass elements may be established through the openings *e* in the disks or they may be shut off from each other by the solid part of the disks, *b*. The glass cells and the rubber disks are mounted in a brass frame which holds them firmly aligned under screw pressure. Free gliding of the disks and freedom from leaks and current loops are assured by liberal use of white vaseline. The electrodes consist of silver wire and silver foil electrolytically plated with AgCl. The side vessels *h* serve for the

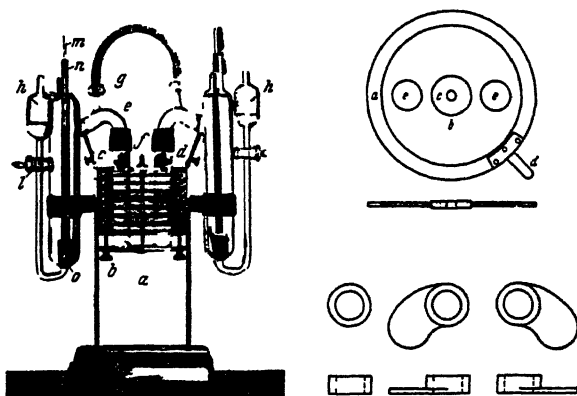


FIGURE 7. Apparatus for Analytical Purposes acc. to Theorell.

storage of KCl solution previous to stratification of the latter at the bottom of the electrode vessels.

A suitable working potential for the apparatus is one of 220 V. which may be taken from the mains provided that the frequent fluctuations are smoothed out by voltage regulator lamps (iron wires in a hydrogen atmosphere). A suitable current intensity is one of about 5 milliampere. The current may of course also be drawn from radio B batteries. Usually the heat effect is kept below 5×10^{-3} watt/cm² but this limit may be somewhat exceeded, if necessary, without serious consequences. This latitude is afforded by the fact that the maintenance of sharply defined boundaries during the electrophoresis experiment is not necessary in Theorell's apparatus since no optical analysis is performed.

For the details of the manipulation of the apparatus reference is made to the paper by Theorell.²⁰ The essential feature of the procedure is the filling of the cell to a certain level with the sol to be studied, all disks being set to permit free contact between the various

glass segments, and the subsequent filling of the remainder of the apparatus with buffer solution of the same pH and conductivity. The lumen of the U-tube is kept open during the electrophoresis. At the conclusion of the run the individual glass cells are shut off by revolving the hard rubber disks and the contents of the cells are successively removed for analysis. The electrophoretic mobility of the substance under investigation is calculated by means of the formula

$u = \frac{x \cdot \kappa \cdot q}{i \cdot t}$, where κ the conductivity in terms of reciprocal ohms, q the cross section of the tube in cm^2 , i the current intensity in amps, t the time in seconds, and x the distance of migration of the colloid in cm. This distance is obtained as the mean of the migration of the substance in the anode and in the cathode limb of the apparatus $\left(x = \frac{x_1 + x_2}{2} \text{ cm}\right)$.

If sufficiently specific methods of analysis are used the mobility of the individual components of a mixture may be determined without previous chemical separation.

Probably the greatest asset of the multi-segment type of electrophoresis apparatus is the possibility of obtaining a sufficient number of different samples for analysis so that important physical-chemical properties of the colloid (or crystalloid) under investigation, *e. g.* electrical mobility at varying pH, position of the isoelectric point, etc. may be determined if its presence can be detected by a suitable test, even when its absolute concentration is very small and the degree of purity quite low. The application of this apparatus to a number of important biochemical problems has been attended by an equal number of successes. The most spectacular one thus far has been the preparation, in pure form, of the yellow oxidation enzyme of Warburg by Theorell. The chemical purification procedures employed by Warburg and Christian (*cf.* Theorell²¹) had led to the removal of other pigments but had not been able to separate the enzyme from large amounts of highmolecular impurities such as proteins and polysaccharides. As a matter of fact the question whether the bearer portion of the enzyme complex was a protein or a polysaccharide was still debated when Theorell applied himself to the problem. The isoelectric point was found at pH 5.15 and electrophoresis on the acid side of this point yielded a purified enzyme preparation with a nitrogen content of 16 per cent, which proved the protein nature of the catalyst. While large-scale purification was only possible after the larger apparatus had been constructed (see below) it was possible to obtain small

²¹ Theorell, H. *Ergebnisse Enzymforschung* 6: 111. 1937.

amounts of the pure yellow enzyme already with the aid of the present small cell. Thus the riboflavin concentration could be increased from 0.01 per cent to 0.3 per cent. The impurities which could be eliminated were: an electroneutral colloid, probably a polysaccharide (50%), a colloid migrating to the anode at any pH (40%), and a nitrogen containing substance with anodic migration at pH 4.65 (6%) (Theorell^{21, 22}).

Other examples of the usefulness of the apparatus are the separation of the protein carrier of the hexosemonophosphate dehydrogenase (Warburg's "zwischenferment") from concomitant hemoglobin (Theorell²³); the characterization of the coferment of this dehydrogenase as a phosphoric acid ester at a time when only relatively crude preparations were available of the substance which was later identified as a triphosphopyridine nucleotide (Theorell²⁴); the subdivision of the hematopoietic liver principle into a thermostable component (R-factor), producing reticulocytosis and replaceable by thymonucleic acid, and a thermolabile (E-factor) necessary to cause erythropoiesis (Eisler, Hammarsten and Theorell²⁵); the control of chemical purification procedures aiming at the isolation of the antigenic substance produced by diphtheria bacilli in the culture medium (Theorell and Norlin²⁶); the electrochemical study of cytochrome *c* (Theorell²⁷).

It is of interest to note that the apparatus may also be used for *diffusion* measurements. This is done by filling the three lower compartments of one limb with the solution to be measured and the three upper cells with water. Only one disk is interposed at the level where the solution and the solvent meet. After temperature equilibration the disk is rotated to permit the formation of a sharp boundary. At the end of the experiment all segments are shut off by the corresponding disks and the contents are analyzed. Two diffusion experiments may be conducted simultaneously in the two limbs of the apparatus. It is important to keep the temperature constant within narrow limits in such experiments. Theorell²⁸ has used the apparatus in this way for the study of the diffusion of the coferment of the hexosemonophosphate dehydrogenase of Warburg and Christian. He concluded on the basis of his results that the coenzyme is a tetrabasic acid and probably a diphosphoric acid ester. The mathematical treatment of such diffusion experiments will be found in his paper.²⁸

²² Theorell, H. *Naturwissenschaften* 22: 289. 1934. *Biochem. Z.* 272: 155. 1934.

²³ Theorell, H. *Naturwissenschaften*, 22: 290. 1934.

²⁴ Theorell, H. *Biochem. Z.* 275: 30. 1934.

²⁵ Eisler, B., Hammarsten, E., & Theorell, H. *Naturwissenschaften*, 24: 142. 1936. *Skand. Arch. Physiol.* 77. 1937.

²⁶ Theorell, H., & Norlin, G. *Z. Immunitäts.* 91: 62. 1937.

²⁷ Theorell, H. *Biochem. Z.*, 279: 463. 1935. 285: 207. 1936.

²⁸ Theorell, H. *Biochem. Z.* 275: 19. 1934.

Interesting experiments on extracellular proteases and crystalline secretin have recently been performed by Ågren and Hammarsten²⁹ with Theorell's apparatus. In a crystalline pepsin preparation made according to Northrop at least two protein components could be demonstrated. Trypsin and carboxypolypeptidase show the behavior of undissociated molecules when subjected to electrophoresis in 30 per cent glycerol solution, *i. e.* they remain stationary in the electric field. When studied in aqueous solutions crystalline carboxypolypeptidase prepared according to Anson shows strongly acidic character and may be dissociated into at least three separate enzymatic components. Purification from tryptic activity may be accomplished by adding protamine which migrates as a base and which will act as a carrier for the tryptic component when present in large excess.²⁹

Ågner³⁰ has recently used Theorell's small-scale apparatus for testing the purity of horse liver preparations which had been obtained by ammonium sulfate fractionation. Upon analysis of the contents of the various compartments it was found that the N- and Fe-concentrations were both proportional to the activity of the enzyme solution in the cells. The copper present in the enzyme preparation also accompanied the enzymatic activity upon electrophoresis with the exception of one cell where the Cu content was twice that usually found.

The modifications recently proposed by Meyerhof and Möhle³¹ are primarily aimed at mechanical improvement of the original Theorell apparatus for analytical purposes. A longitudinal section through the improved arrangement is shown in FIGURE 8.

In modifying certain mechanical features of the original apparatus the authors strove (1) to mount the cell as stably as possible to eliminate risks arising through vibrations, (2) to put the glass segments under an even pressure and to improve their fit so as to prevent leaks and current loops and (3) to facilitate the filling of the apparatus and the manipulation of the shutter disks from without the tank in order to avoid convection currents. The temperature was maintained constant at 20° within 0.001° with the aid of a slow stream of cooling water and a gas microburner equipped with a gas pressure regulator and controlled by a toluene thermo-regulator. As a source of current a radio B battery of 100 V. was used as an additional safeguard against vagabond currents.

²⁹ Ågren, G., & Hammarsten, E. *Enzymologia* 4: 49. 1937. *Jour. Physiol.* 90: 330. 1937.

³⁰ Ågner, K. *Biochem. Jour.* 32: 1702. 1938.

³¹ Meyerhof, O. & Möhle, W. *Biochem. Z.* 294: 249. 1937.

Meyerhof and Möhle³¹ have employed this apparatus in electrophoretic investigations on important components of the fermentation systems of yeast and muscle tissue. The isoelectric point of cozymase was found at pH 3 and the electrotitration curve could be duplicated by plotting the electric mobility curve. When examining hexahydrocozymase an additional weak acidic dissociation constant could be

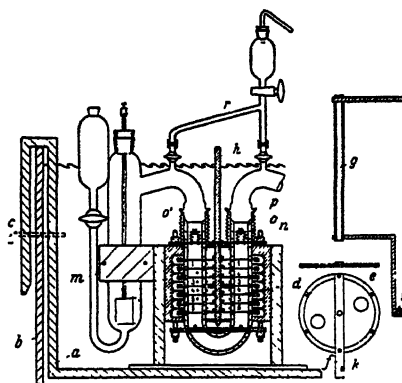


FIGURE 8. Modification of Theorell's Small-scale Apparatus by Meyerhof and Möhle. a, Brass frame of the apparatus to fit the wall of the thermostat; b; c, pins to secure position of cell holder; d, hard rubber disks (0.2 mm) mounted in brass rings; e; f, brass strip across disks to serve as lever; g, bent metal tool for turning disks from above; h, extension of central axis to carry the tool g; i, small pin to fit hole, k, in lever of disks; m, brass holder for electrode vessels; n, brass bridge for holding the glass segments of the U-tube together under pressure; o and o', springs attached to hooks, p, of U-tube and to hooks of glass joints of side arms; r, arrangement for equalization of hydrostatic pressure in apparatus. The glass rings forming the limbs of the U-tube are ground and polished like optical flats. The right electrode vessel and other parts of the apparatus which is symmetrical with respect to the axis have been omitted in the drawing. For greasing the glass rings and the hard rubber disks lanoline was found superior to vaseline.

detected. The enzyme hexokinase as prepared by CO₂ precipitation of yeast maceration juice has an isoelectric point at pH 4.9 whereas the enzyme obtained from autolyzed yeast shows a slightly different electrophoretic behavior. Zymohexase, the enzyme in muscle extracts catalyzing the equilibrium reaction Hexosediphosphoric acid \rightleftharpoons 2 Dihydroxyacetonephosphoric acid, has an isoelectric point of pH 6.9. The pH-mobility curve has a much smaller slope on the alkaline than on the acid side of the isoelectric point.

Apparatus for Preparative Purposes

Although the apparatus described in the preceding section was developed primarily for analytical purposes it may also be used for the

purification of colloids. However, the volume of the individual glass cells (2 ccm) is too small to lend itself to preparative operations on a practical scale. In principle a cylindrical U-tube is not very suitable for preparative purposes because the diameter of the tube is limited to small dimensions if adequate cooling even of the central portion is to be safeguarded. The second apparatus of Theorell³² for preparative purposes is therefore designed along different lines (FIGURE 9).

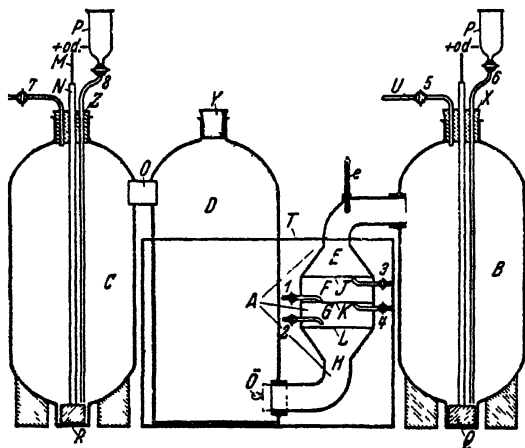


FIGURE 9. Theorell's Apparatus for Preparative Purposes. A, Separation chamber; B and C, Electrode vessels (10 liter); D, Intermediary Chamber (10 liter); E and H, communication pieces; F and G, glass cylinders (10.5 cm. diameter, 4 cm high); J, K, and L, Membranes, two of which (J and L) are impermeable for the colloid to be purified while K is permeable for it; 1, 2, 3 and 4, taps for admitting and withdrawing fluids; P, storage vessels for saturated KCl solution; T, thermostat to be filled with ice or flushed with cold water, equipped with glass windows; Source of current: Alternating current obtained from the mains (220 V.) is transformed into a current of 3000 V. by a transformer (1000 watt maximum) and then rectified by a tube capable of handling a maximum of 3000 V. and 3 Amp.

The principle of the apparatus is as follows. The colloid to be purified is permitted to migrate in the electric field from one compartment (F) into another (G) through a permeable membrane. The colloid is prevented from leaving the second cell by an impermeable membrane. While it thus accumulates in this chamber, the colloidal impurities remain in the first cell if the experimental conditions are correctly chosen. The yield is almost quantitative in a relatively short time provided that a sufficiently high voltage is applied. The electrodes are reversible and the current passes through a buffer solution of the same pH and conductivity as the solution of the colloid. In selecting the membranes not only the permeability towards the

³² Theorell, H. *Biochem. Z.* 278: 291. 1935.

colloid but also their effect on the hydrogen ion and salt concentration within the compartments F and G must be considered. Their nature will probably vary from case to case depending on the nature of the substance to be purified and on the experimental conditions. It is noteworthy that for preparative purposes it is not necessary to maintain a constant and low temperature throughout the entire apparatus. Thus, when using a buffer solution of a conductivity of $\kappa = 0.34 \cdot 10^{-3}$ recipr. ohms and working at about 2000 V. and 0.2 Amp, the temperature in the bend of E as read on the thermometer e (FIGURE 9) may rise to 60 and 70° without interfering with the experiment. With adequate cooling in T, the temperature in the separation cells F and G will remain below 10° when a potential gradient of 6 V./cm, which is suitable for rapid electrophoretic separation, is applied. In practice it is not feasible to maintain a perfectly constant pH and conductivity in all parts of the apparatus during the experiments, but minor changes have been found to be harmless by experience.

Thus far, the apparatus has been mainly employed in the large-scale purification of the yellow oxidation enzyme of Warburg and Christian from bottom yeast. The term "large-scale" is to be taken as *relative* only, when comparing the capacity of the glass segments of the present apparatus (350 cc) with that of the apparatus discussed above (2 cc). Theorell^{21, 22} was able, with the aid of the apparatus, to remove large amounts of contaminating polysaccharides from preparations of the yellow enzyme, a finding that was later confirmed by Kuhn and Rudy.²³ In order to eliminate the last traces of associated foreign proteins, however, Theorell found it necessary to subject the electrophoretically purified yellow enzyme to ammonium sulfate fractionation while Kuhn and Rudy employed adsorption on aluminum hydroxide gel for this purpose. After a number of trials Theorell determined the following conditions as optimal for electrophoretic purification of the enzyme: Acetate buffer of pH 4.1 and $\kappa = 0.34 \cdot 10^{-3}$; hardened filter paper (Schleicher and Schüll) as material for the permeable membrane, K; genuine parchment paper as material for the impermeable membranes, J and L; current intensity of 0.16 Amp; length of electrophoresis 14 hours. It should be mentioned that the isoelectric point of the enzyme is at pH 5.2 and that its molecular weight is about 80,000. Attempts to substitute for the filter paper sintered glass filter plates as permeable membranes and for the parchment paper cellophane or albumin-collodion sheets as impermeable membranes were unsuccessful in the present case. These membranes gave rise to disturbances due to endosmosis and gross pH- and conductivity changes.

²³ Kuhn, E., & Rudy, H. Ber. 69: 1974. 1936.

Recently the apparatus has also been applied to the purification of cytochrome *c* (Theorell²⁷). Cytochrome preparations from beef heart muscle which had been chemically purified by ammonium sulfate fractionation could be obtained completely pure by electrophoresis. It was found best to work with buffer solutions of pH 9, where the cytochrome migrates towards the cathode (isoelectric point pH 9.8) while the protein impurities will all migrate towards the anode at this high pH. By inserting a filter paper diaphragm the small-scale apparatus (see above) could be used for the electrophoretic purification of small quantities of cytochrome. Recently Theorell has been able to raise the iron content of cytochrome *c* from 0.34 to more than 0.4 per cent by an electrophoretic procedure (unpublished).

ELECTROPHORETIC STUDIES WITH THE NEW APPARATUS AND METHODS OF TISELIUS

The moving boundary method of electrophoresis has derived what might prove to be its greatest stimulus in its history from the recent experimental contributions made by Arne Tiselius. In continuation of his earlier work on the electrophoresis of proteins which has already been discussed above, Tiselius has designed new equipment and he has applied new methods of optical analysis to the moving boundary method. With the aid of these new and efficient methods he and his co-workers have been able to obtain results within the past two years the importance of which can hardly be underestimated. In his hands the method has experienced such a swift evolution towards utmost refinement that many workers in the field are inclined to rate the method of electrophoresis as an even more specific and powerful tool for the sorting of protein molecules than the method of ultracentrifugation which has been developed in the same institute under the leadership of The Svedberg.

The Separation Apparatus of Tiselius

A schematic longitudinal section through the new apparatus is shown in FIGURE 10. (Tiselius¹⁴.)

The apparatus is composed of a central U-tube part connected with rubber tubing through intermediary glass pieces with the electrode vessels the volume of which depends on the duration of the proposed experiment (0.5 to 2 liters). The electrode vessels contain reversible Ag-AgCl electrodes surrounded by a bottom layer of saturated KCl

solution as in the earlier Tiselius cell.^{22a} The U-tube portion itself is built up of *rectangular* glass cells which are made from parallel glass plates, cemented with acid-proof silicate cement. The bottom part was originally rounded like an U but has been replaced subsequently by a flat rectangular cell. All sections of the U-tube, including the top part serving for the connection with the electrode tubes, are cemented to comparatively large precision-ground glass plates which are well greased to permit free gliding. Tiselius recommends vaseline thinned with paraffine oil (2 parts to 1) for the purpose. In this

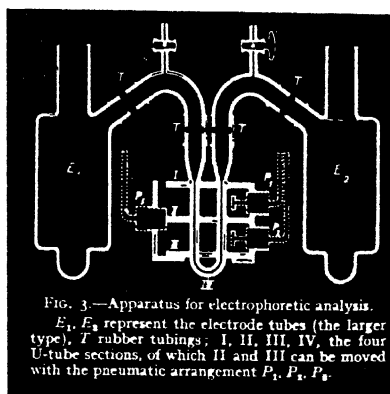


FIGURE 10. Tiselius' New Apparatus for Electrophoretic Separations

laboratory "celloseal," a new proprietary lubricant, is used to advantage because it shows very little change in viscosity with temperature in the range from 0 to 25°. The glass sections are held together in a metal stand under slight spring pressure. The arrangement resembles in certain respects the diffusion apparatus of Cohen and Bruins. The sections are moved by a pneumatic arrangement (shown by the dotted lines in FIGURE 10) which is actuated by a small air pump. A slight motion of the sections shuts them off from each other or, when reversed, aligns them in a precise manner.

The separation cells so far made have been of three different sizes. The most extensively used type is one accommodating 11 cc. of the sol to be studied in the two lower glass compartments plus the bottom piece. The internal cross-section of these cells is 3 × 25 mm, each

^{22a} The latest model has a V-shaped cemented bottom piece which, contrary to the flat cell, permits the passage of protein boundaries without mechanical disturbances.

cell holding about 4 cc. of fluid. More recently (Tiselius³⁴) a micro-apparatus for 2 cc. solution and a macro-apparatus for 150 cc. solution have been described.

In electrophoretic fractionation studies the following considerations (Tiselius³⁴) must be taken into account.

"Consider two components, *A* and *B*, with the mobilities u_A and u_B migrating in the same or in opposite directions in an electrophoresis tube of cross section area q cm². The plus sign indicates a positive charge for u_A and u_B , and *vice versa*. If the current is i amp and the conductivity K ohm⁻¹, the potential gradient in the tube is $i/q K$ volt/cm and the boundaries will move with the velocities $i u_A/q K$ and $i u_B/q K$ cm per sec. After a time t partial separation has taken place, so that, diffusion being neglected, the volume in cc. on each side containing pure *A* or *B* is

$$\frac{i(u_A - u_B) t q}{q K} = (u_A - u_B) \frac{i t}{K}.$$

Independently therefore of the dimensions of the apparatus, a given degree of separation corresponds to a certain amount of electricity sent through. It is assumed that reversible electrode tubes of some sort are used, and that the ions surrounding the electrodes have a mobility of u . When the quantity of electricity $i x t$ is sent through, these ions similarly sweep through a volume in cc. of $u x \frac{i t}{K}$. If the volume in cc. of colloidal solution to be separated is v , there must be a volume of at least $\frac{u}{u_A + u_B} x v$, between the electrodes and the boundaries, otherwise the ions originally present at the electrodes would reach the boundaries. In practice the volume should be about twice as large, since diffusion also interferes. If we desire to separate, say, 10 cc of two components with a difference in mobility of 0.5×10^{-5} , using reversible KCl(AgCl)Ag electrodes, the mobility of K and Cl being 65×10^{-5} , the calculated electrode vessel volume would be $\frac{65}{0.5} \times 10 = 1300$ cc, and that used should be about 2600 cc."

It would be expected that for the macro-cells very large electrode vessels would be required in order to prevent appreciable changes in electrolyte distribution throughout the apparatus in experiments of long duration. It has now been found (Tiselius^{34, 35}) that electrode

³⁴ Tiselius, A. Kolloid Z. 85: 129. 1938.

³⁵ Tiselius, A. Svensk. Kem. Tid. 50: 58. 1938.

flasks of ordinary size may be used if a layer of concentrated buffer solution is interposed in the electrode vessels between the KCl solution at the bottom and the supernatant dilute buffer which has been equilibrated by dialysis against the colloid solution. The boundary between the concentrated and the dilute buffer solutions will remain almost stationary even when current is sent through the cell for a long time. The main difficulty in preparative work, however, particularly with fairly concentrated sols are the changes in ionic concentration which occur at the interface between the solution of the colloid and the supernatant buffer. They give rise to false boundaries (so-called δ -boundaries, see below), to changes in H-ion concentration and other anomalies which are still being investigated in Upsala and other laboratories.

Probably the most ingenious feature of the new apparatus of Tiselius is the so-called "*compensation movement*," the theory of which he described as follows.¹⁴

"When both substances migrate in the same direction the possibility of sufficient separation is limited by the fact that long before the desired separation has been reached, both substances have migrated out of the electrophoresis tube. This is a somewhat serious limitation, since the absolute differences in mobilities are very often much larger at pH regions where both components have mobilities of the same sign (*e. g.* the serum proteins). Moreover, lack of solubility often prevents a choice of a pH between the isoelectric points. For this reason we arranged for a slow and uniform movement of the solution in the electrophoresis tube at an exactly known rate and in a direction opposite to the migration, by slowly lifting a cylindrical glass tube by clockwork out of the liquid in one electrode tube during the electrophoresis. If the rate at which the tube is lifted is l cm per hour, its cross-section area p cm², the free surface of the liquid in each electrode tube Q cm², and the cross-section area of the electrophoresis tube q cm² then a movement of a given level in this tube will take place, at a rate of $\frac{l p Q}{q (2 Q - p)}$ cm per hour. By suitably choosing l and p any desired rate can be obtained; even in the narrow tubes used in our apparatus a rate of several centimetres per hour did not markedly blur the boundaries in the electrophoresis tube. For fractionation purposes, this "*compensation movement*" is adjusted so that the observed boundary separating two fractions obtains a suitable "*apparent mobility*" and consequently, at the end of the run, the column of the solution can be cut off exactly at the right place. . ."

The compensation movement may be used in several ways. Thus, the shift in the level of the boundary of the colloid due to electrophoretic migration may be offset by the change in hydrostatic pressure caused by the compensation drive. In this case the colloid will remain stationary in the U-tube, it will "mark time" while impurities of a higher mobility will migrate out into the upper compartments and impurities of a smaller mobility will be drawn up into the opposite compartments by the action of the compensation movement. Whereas the actual height of one compartment in the medium-size apparatus is only 4 cm, the effective path of electrophoretic purification amounts to 8 cm under these conditions. Another application of the compensator which has been particularly stressed by Tiselius consists in adjusting the compensation movement to a rate corresponding to the arithmetic mean of the mobilities of two colloids to be separated. At this speed the faster component will still be able to continue its electrophoretic migration in the original direction, although at a smaller rate, whereas the slower of the two components will be pulled back by the compensation drive, thereby ever increasing the degree of separation of the two colloids. This makes it possible to work at a pH where both colloids have a high mobility of the same sign and still to end up with one of the components in the right hand limb and with the other in the left hand limb of the cell. The compensation drive has aptly been compared with a treadmill. It extends the separation capacity of the apparatus to its fullest extent.

In practice the compensation movement may be brought about by various devices. Tiselius uses a clockwork with variable speed, Stenhagen³⁶ has injected buffer solution slowly into one of the electrode vessels in order to bring a protein-buffer boundary out from behind the cell end-plates before the current was switched on, and Longworth³⁷ employs a synchronous motor with a set of exchangeable transmission gears driving a syringe. The simple compensation mechanism used in the writer's laboratory consists of an inexpensive alarm clockwork where the hour hand has been replaced by an aluminum pulley with ten individual steps for varying the rate of lifting a rod of insulating material out of one electrode vessel. This arrangement, when used in conjunction with a rod of insulating material of 16 mm. diameter, permits a boundary displacement of 7 mm to 32 mm per hour depending on the pulley step used. These rates of compensation cover adequately the range desired when working with potential

³⁶ Stenhagen, E. *Biochem. Jour.* **32**: 714. 1938.

³⁷ Longworth, L. G. Private communication.

gradients of 5 to 15 V./cm and with a number of biologically occurring protein mixtures, *e. g.* blood sera, yeast maceration juice, etc. Fine adjustments are made by varying the voltage applied to the apparatus.

The resolving power of an electrophoresis apparatus is limited by the magnitude of the potential gradient which may be applied without causing appreciable thermal convection currents due to the Joule's heat developed within the cell. In the earlier, cylindrical electrophoresis cell of Tiselius the maximum load had been found to be about 0.01 to 0.02 watts per cc. Convection currents are more readily produced in wide than in narrow tubes. The problem has been carefully studied by Tiselius^{2, 14}. Given a load of w watts/cc. a thermal conductivity of the solution examined K and the temperature t , the temperature gradient dt/dr at the distance r from the centre of a cylindrical tube becomes $dt/dr = w r/2 K$, *i. e.* proportional to the radius of the tube. The temperature difference between the mid-point and the periphery of the fluid column in the tube is $t_c - t_o = \frac{w r^2}{4 K}$.

This makes it theoretically very desirable to employ tubes of as small a diameter as feasible. On the other hand, there is a lower limit imposed by other considerations. Thus, upon decreasing the internal diameter of a cylindrical tube, *e. g.* to 3 mm, the retarding effect of the walls on the flow of the solution increases very rapidly and may cause blurring of the boundaries (Tiselius²). Furthermore, the volumes which may be handled with such a cell would become too small to be of use in preparative experiments. However, by using *flattened* tubes large volumes may be accommodated with satisfactory heat conduction. The new rectangular glass cells of Tiselius¹⁴ are the outcome of these considerations. Furthermore, since the thermal convection depends on the ratio dp/dt which becomes zero in the case of water and dilute aqueous solutions at 4°, *i. e.* at the density maximum, adjustment of the temperature to 4° during electrophoresis ought to assist materially in suppressing convection phenomena. Tiselius found in actual experiments that when the bath was held close to 0°, heat loads of 0.5 to 1.0 watt per cc. are tolerated by flattened tubes. While it is true that lowering the temperature will correspondingly decrease the rate of electrophoretic migration, the diffusion of the protein boundary is also reduced so that the quality of the separation is not affected.

The electrophoresis cell is immersed in a well-insulated water thermostat of about 100 liter capacity. For maintaining a temperature of the bath just above 0° Tiselius recommends either the use of an electrolux gas refrigeration unit of 100 kcal/hour capacity, in heat

exchange with the tank through a secondary circulation system with alcohol as carrier fluid, or a motor driven compressor aggregate controlled by a contact thermometer. The heat developed in electrophoresis experiments is stated to exceed rarely 20 to 30 kcal. per hour.

As a source of current Tiselius recommends an arrangement consisting of a centre-tapped 2×1500 V. transformer (capacity 0.1 amp) and two half-wave mercury rectifying tubes of a maximum peak inverse voltage of 7500 V. and a maximum peak plate current of 0.6 amp. A filter circuit containing a 20 Henry choke and a $4 \mu F$ condenser is used. The voltage fluctuations are small if the primary voltage is kept constant with an induction regulator. In the writer's laboratory radio B batteries (layer-built dry cells of 45 volts each) have been found satisfactory for the supply of current up to 600 V. and 20 milliamperes.

The "dissection" of the fluid columns after electrophoresis may be carried out either by trapping the portions containing the various protein fractions in different compartments of the new separation apparatus with the aid of the pneumatic arrangement (indicated in FIGURE 10 by dotted lines and by removing them with capillary pipettes or by using a convection-free pipetting device. The pipette has a broad opening with a fused-on sintered glass filter disk which distributes the stream of liquid over an area as large as possible. A water-manometer arrangement creates the right degree of suction for removing successive layers of fluid from the column at levels sought with a rack and pinion drive. (Tiselius³⁴.)

Methods of Optical Analysis

It would hardly be practical to follow the migration of the boundaries in the new Tiselius apparatus with the light absorption method. When working with colorless proteins it would be necessary to have all parts in the light path made of quartz of the highest quality, including the rectangular separation cells. The cost of such an arrangement would be prohibitive.

It is one of the great contributions of Tiselius to have adapted the so-called schlieren method of A. Toepler for use in electrophoretic experiments. Inasmuch as the theory of the schlieren method and its application to electrophoresis are fully discussed by Dr. Longworth in his contribution to this symposium only little need be said about the subject here. Tiselius, in his publications describing his new apparatus, mentions the principle only very briefly and he refers for all details to the text books of practical physics and to the monograph on

the subject by Schardin.³⁸ Those who desire to construct a Tiselius electrophoresis apparatus in their own workshops are likely to experience the same disappointment as the writer when searching for detailed information on the arrangement of the optical tract and on the properties and construction of the individual parts in the otherwise excellent treatise of Schardin or in textbooks on practical physics, *e. g.* that of Kohlrausch. On the other hand, considerable help was obtained by consulting the original paper by Toepler,³⁹ written in 1867, where the subject is treated in a lucid and thorough manner.

Tiselius¹⁴ states that a Dallmeyer objective of f about 60 cm with an aperture of 10 cm was placed as near to the electrophoresis cell as possible. A lamp and a diaphragm are placed at about 100 cm distance, on the same side of the thermostat. The image of the diaphragm is projected on the other side of the thermostat, at a distance of about 100 cm from the window. Here a projection lens is placed which is partially covered by a vertically movable screen with a sharp edge. Instead of the projection lens a camera objective may be used in order to obtain photographs of the boundaries.

The arrangement used in the writer's laboratory is schematically shown in FIGURE 11.

The lenses incorporated into our apparatus are corrected for spherical and chromatic aberration, eliminating the need for light filters. Their focal lengths are not optimal (the lenses formed parts of dismantled electrocardiographic equipment) but the main condition for analysis by the schlieren method, viz. that the schlieren lens, E , must reproject the edge of the first schlieren diaphragm (close to the light source) in the plane of the second, movable schlieren diaphragm (in front of the camera lens) is fulfilled. On the other hand, the camera lens, F , is focussed on the U-tube of the electrophoresis cell, with a magnification factor of 0.4. In actual practice this has not been found a serious draw-back while the lens combination and the distances chosen have permitted the accommodation of the entire apparatus on the floor space available (over-all length, 450 cm).

When the movable schlieren diaphragm approaches the reprojected slit image from above, the main image is blocked before the deviated or secondary images due to the boundaries in the electrophoresis cell are affected. In this case the boundaries appear on the matt screen of the camera as brilliant white lines on a dark background. Vice

³⁸ Schardin, H. Das Toeplersche Schlierenverfahren. VDI-Verlag, G. M. B. H., Berlin, 1934.

³⁹ Toepler, A. Poggendorf Annalen 131: 33. 1867. See also Logsworth, L. G. & MacInnes, D. A. Chem. Reviews 24: 271. 1939.

versa, when the schlieren diaphragm is moved upwards from below the reprojected slit image, the boundaries appear as black lines on a light background. Either method has certain advantages making it desirable to make provision for both types of schlieren observation. The imaging of the boundaries as black lines on a white background has the advantage that the edge of the metal shield of the electrophoresis cell may be used as the reference point for mobility measurements

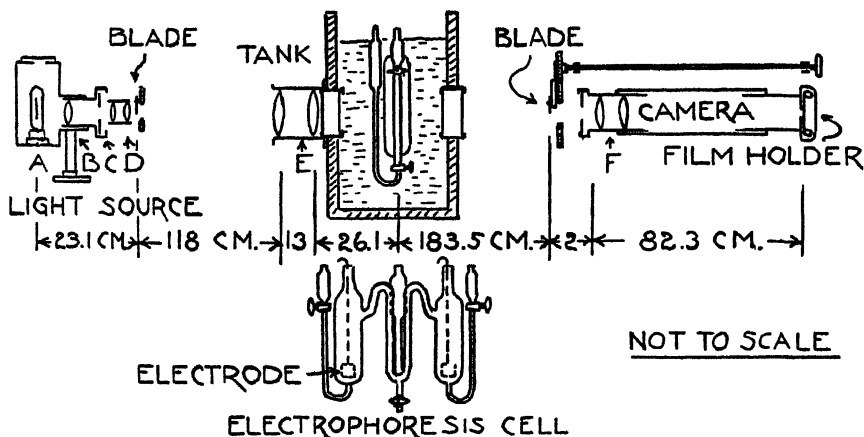


FIGURE 11. A, Mazda Biplane Filament Projection Bulb; B, Condenser Lens, plano-convex, 60 mm diameter, 40 mm focal length; C, Iris Diaphragm, closed down to about 2 mm opening; D, Condenser System, formed by two plano-convex lenses of 45 mm diameter, 100 mm focal length; "Blade," First Schlieren Diaphragm, consisting of a circular fixed diaphragm of 7 mm diameter and of a sharp edge crossing the opening in the upper third (In a later arrangement, D has been omitted and a diffraction slit with 30 mm long jaws has been substituted for the "Blade"); E, Schlieren Lens, 22×20 Rectilinear (R. & J. Beck), 95 mm diameter, 762 mm focal length, distance between the two component lenses 130 mm; "Blade," Second Schlieren Diaphragm, consisting of a fixed circular opening of 28 mm diameter and of a horizontal sharp edge movable across the fixed opening by a remote mechanical control mechanism; F, Camera Lens, 75 mm diameter, 390 mm focal length, distance between the two component lenses 75 mm (Voigtlander).

and furthermore that there can be no doubt as to the number of individual boundaries visible. The reverse method, on the other hand, frequently yields somewhat better defined boundary images. The inherent high sensitivity of the method calls for a mechanism capable of a delicate adjustment of the position of the mobile schlieren diaphragm or blade.

The schlieren method is particularly suited for the determination of the electrophoretic mobility of proteins, for ascertaining the number of components in protein mixtures and of their degree of molecular dispersion. Depending on the dimensions of the apparatus, and on the absolute and relative concentrations of the various components

each individual component will give rise to an individual boundary or schlieren upon resolution of the mixture in the electrical field. The lowest concentration in which a protein can be detected optically in Tiselius's apparatus, employing the 11 cc. cells, is about 0.03–0.05 per cent. The relative amount of a protein impurity which may thus be detected in a protein preparation will therefore vary with the total protein concentration employed. If the latter amounts to 1 gm. per 100 cc solution an impurity present in about 5 per cent may be spotted provided that the experimental conditions allow for sufficient separation in the field. Furthermore a homogeneous protein component will yield a sharply defined boundary throughout the run whereas a spreading of the boundary during electrophoresis indicates electrochemical heterogeneity provided that mechanical vibrations, thermal convection currents and diffusion of the protein to an appreciable extent are avoided. The rate of migration of a boundary may either be determined by visual observation of the movement of the boundaries relative to a scale placed in the focus of the camera or by photographing the boundaries in suitable intervals and measurement of the change in distance with time relative to a reference line. The degree of definition of the boundaries seldom permits to use comparators of an accuracy exceeding 0.01 mm. Usually it is sufficient to measure the distances with a magnifying eye-piece equipped with a transparent scale reading to 0.1 mm as used for the inspection of line spectrograms.

Experience in our laboratory has shown that mobility values may be determined and reproduced with an average accuracy of about 5 to 10 per cent. This is usually sufficient to "label" a protein when controlling the result of chemical fractionation procedures by electrophoretic analysis.

Recently the well-known "refractive index" or "scale" method which has proven so successful in ultracentrifugal analysis in Svedberg's institute has also been applied to electrophoresis. The theory and practice of this very accurate and sensitive method are fully dealt with in Lamm's⁴⁰ paper on the study of starch by means of diffusion and of the analytical ultracentrifuge. The adaptation of the scale method to electrophoresis does not present any difficulties. The main advantage of the scale method over the Toepler schlieren method appears to consist in the fact that the evaluation of the electrophoretic diagrams thus obtained permits the determination of the absolute and relative concentrations of the various components

⁴⁰ Lamm, O. *Nova Acta. Soc. Sci. Upsala* IV, 10 (6), 1938.

of a mixture throughout the area under observation in addition to the other types of information which may also be gained by the schlieren method. A drawback of the scale method is that the final result may only be arrived at after a large series of precise comparator readings and after tedious arithmetic operations.^{40a} Examples for the application of the scale method to electrophoretic studies will be found in the papers by Tiselius and Horsfall⁴¹ and Tiselius and Kabat.⁴²

Assembly of Tiselius' Apparatus

The detailed construction of the separation cell mechanism and the general features of the entire set-up have been described by Tiselius. The construction of the apparatus in the form in which it is commercially available is depicted in FIGURE 12.

In the apparatus constructed and used in the writer's laboratory,

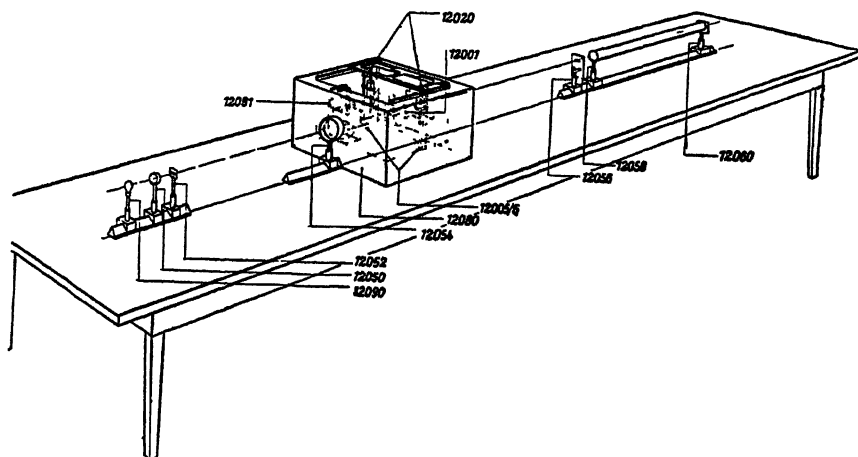


FIGURE 12. Arrangement of the new Tiselius Apparatus.

^{40a} The recently developed modification of the Toepler schlieren method by Longworth (*J Am. Chem. Soc.* **61**: 529, 1939) is capable of yielding results of the type secured by the scale refractive index method but with considerably less effort. In this modification the blade or movable schlieren diaphragm is lowered or raised mechanically while the plate or film is drawn past a fine slit at a rate which is maintained at a constant ratio to the speed of the movement of the schlieren diaphragm. In this way the entire refractive index gradients existing in the electrophoresis cell are automatically scanned and photographically recorded. The areas under the contours of the curves thus obtained are proportionally to the concentration of the colloid components giving rise to the gradients. This ingenious method has been successfully applied in the writer's laboratory to the study of blood sera of several species and under varying conditions of a pathological and immunological nature (unpublished).

⁴¹ Tiselius, A., & Horsfall, F. L. Jr. *Jour. Exp. Med.* **69**: 83, 1939.

⁴² Tiselius, A., & Kabat, E. A. *Jour. Exp. Med.* **69**: 119, 1939. *Science* **87**: 416, 1938.

the tank with a capacity of about 100 liters is thermally insulated by rockwool. It is kept close to 4° by an Electrolux gas refrigeration unit of 100 kcal/hour capacity, connected with the tank by a closed circulation system filled with 95 per cent alcohol, and a thermostatically controlled electrical heating system. The motor-driven stirrer and the circulation pump are mounted on a Lally column resting on a sheet of rubber packing in order to prevent the transmission of vibrations to the electrophoresis cell. The light source and the camera are mounted in a similar manner. The light source is a 300 watt biplane filament projection lamp, contained in a Bausch & Lomb microscope lamp equipped with an iris diaphragm and a rack and pinion drive for the condenser lens. The schlieren lens is mounted on a sturdy bracket so as to be close to the double window of the tank (100 mm free diameter). The camera is set on a double optical bench and consists of two lengths of heavy-walled brass tubing turned to yield a sliding fit. Before the camera lens there are mounted on riders an iris diaphragm, the movable schlieren diaphragm and a revolving disc carrying fixed diaphragms of varying shape and size. Following a suggestion of Dr. Longworth the movement of the schlieren diaphragm is effected by a micrometer screw which is coupled to the remote control by a friction wheel and two conical gear wheels. Two different blades may be inserted according to whether the boundaries are to appear as light lines on a dark background or vice versa.

The electrophoretic diagrams are recorded on standard 35 mm positive film contained in a Leica Eldia Printer. The latter fits tightly into a metal casing mounted on a slide which permits a rapid change-over from photography to visual observation on a ground glass screen.

The type of electrophoresis cell employed in the writer's laboratory depends on the aim of the experiment. For purposes of examination of a given protein preparation for the number of principal components present, for the degree of homogeneity of purified fractions, and for determinations of electrophoretic mobility the simple U-tube cell of Tiselius may be used to advantage. The cell consists of Pyrex glass and is made of one piece. The U-tube length available for observation is defined by metal shields of 60 mm open area. The cross-section of the tube is 0.587 cm². When using this cell it is advisable to work with potential gradients of the order of 3 to 4 V./cm, corresponding to an applied potential of 180 to 200 V. and a current strength of 3 to 4 mA. when the ionic strength of the solutions in the cell is adjusted to about 0.1. The advantage possessed by this cell

over the more elaborate separation cell of Tiselius consists in the simplicity of its manipulation. The process of stratification of the protein solution under the buffer solution requires a little practice. On the other hand, under the conditions existing in our apparatus, the images of the protein boundaries obtained with the simple U-tube cell are more satisfactory than those obtained with the rectangular cells. When it is desired to separate the various protein fractions mechanically for chemical and biological examination the new separation cell of Tiselius (FIGURE 10) is employed. With this cell higher potential gradients and current strengths up to 15 mA. may be applied without running the risk of thermal disturbances.

Results obtained with the aid of Tiselius' new apparatus

The results procured with the new apparatus and methods in Upsala by Tiselius and his collaborators may be summarized as follows. The electrophoretic diagram of blood serum which in the analytical ultracentrifuge shows only two to three different molecular species, reveals the presence of four well-defined protein components. One of these is serum albumin while the other three components are globulins. They have been designated as α -, β - and γ -globulin in accordance with their electrophoretic mobilities (Tiselius⁴³). The dependence of the mobilities of the various components upon the hydrogen ion concentration may be gleaned from TABLE 1 which is taken from Tiselius' paper.

TABLE 1

ELECTROPHORETIC MOBILITIES OF THE COMPONENTS IN 4 \times DILUTED NORMAL HORSE SERUM AT DIFFERENT pH VALUES AND CONSTANT IONIC STRENGTH 0.1 ($t = 0^\circ$)

Mobility $\text{cm}^2 \text{V}^{-1} \text{sec}^{-1} \times 10^5$

Buffer	pH	Albumin	Globulin α	Globulin β	Globulin γ	Globulin δ
Acetate	5.05	-1.39	-0.36	-0.2	+1.02	+1.53
Phosphate	6.02	-4.24	-2.76	-2.10	-0.23	+0.4
Phosphate	6.87	-5.39	-3.98	-3.25	-1.20	-0.2
Phosphate	8.03	-7.61	-5.79	-4.57	-1.90	-0.1

The δ -boundary which is practically stationary and which was at first attributed to a fourth globulin component has since been recognized as an anomaly due to changes in concentration of buffer ions occurring upon electrophoresis of relatively concentrated protein solutions (Stenhagen³⁶ Tiselius³⁴). When plasma is used instead of

⁴³ Tiselius, A. *Biochem. Jour.* 31: 1464. 1937.

serum, an additional boundary corresponding to fibrinogen is observed between the γ - and the β -globulin boundaries (Stenhagen³⁶). Schlieren diagrams obtained with human serum and plasma are reproduced in FIGURE 13.

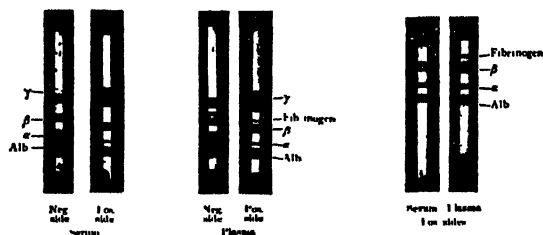


FIGURE 13. Toepler schlieren diagrams obtained upon electrophoresis of human serum and plasma (Stenhagen³⁶).

It is important to note that the various serum protein fractions as obtained by ammonium sulfate fractionation and other chemical procedures are not identical with the fractions separated by electrophoresis: They represent mixtures of the components identified by electrophoretic analysis. Thus the fractions most readily salted out consist mainly of γ and β and of small amounts of α globulin and albumin, whereas the proteins precipitated above 55 per cent saturation with ammonium sulfate contain approximately 75 per cent albumin and only 25 per cent of the globulins. Serum globulin, after having been precipitated 3 times with 55 per cent ammonium sulfate, dissolved in 5 per cent NaCl, dialyzed and electro-dialyzed ("Pseudoglobulin") will contain as much as 75 to 85 per cent globulin α and as the remainder a slow component, probably γ -globulin. So-called "euglobulin" contains more β and γ , and less α globulin. Very probably the solubility differences between pseudo- and euglobulin depend upon the difference in solubilities exhibited by the individual components.⁴³

When passing from dilute to more concentrated solutions a marked change takes place in the appearance of the electrophoretic schlieren diagram. The δ -boundary increases in strength at the expense of the actual globulin boundaries. When undiluted serum is run with 6/7 serum as the supernatant to avoid boundary disturbances the effect is very marked, only the albumin and the δ -boundary are visible with faint indications of the three globulin boundaries. Tiselius assumes that this may be due to the neutralization of the charge of the globulins in undiluted serum by some unknown substance. This neutralizing agent would be dissociated off more or less completely upon dilution, permitting the three globulins to show their individual

migration. This tentative interpretation has since become open to question because Tiselius could show that the δ -boundary does not represent a protein boundary and therefore cannot gain at the actual expense of globulin components. It may be that the anomalies observed when working with concentrated sera are due to unfavorable optical conditions for the Toepler schlieren analysis rather than to true changes in the concentration of the serum components. It must be remembered that the detection of the boundaries is made possible only by the refractive index differences at the interfaces between the individual layers. The α - and β -globulin boundaries are situated within the moving protein column and are liable to be masked or obliterated more readily than the albumin and globulin γ boundaries which border the supernatant buffer solution.

With the aid of the separation mechanism Tiselius was able to obtain small amounts of the various serum proteins in pure state for chemical studies. That they are chemical individuals is born out by the fact that their absorption spectra show distinct differences.^{43a}

Immune sera of the horse and of the rabbit differ markedly from each other and from normal serum. In horse serum the antibody appears as a new component with a mobility intermediate between that of the γ - and β -globulin components. In the case of the rabbit no new boundary appears but the amount of γ -globulin is greatly increased.⁴² FIGURE 14 represents the electrophoretic diagrams obtained

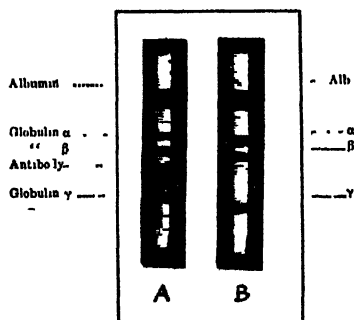


FIGURE 14. Electrophoretic Diagrams of unabsorbed (A) and absorbed (B) antipneumococcus horse serum (Tiselius and Kabat).⁴¹

with unfractionated Type I antipneumococcus horse serum and with the same serum after the removal of the antibody by addition of the

^{43a} Normal human serum has recently been further studied in the Tiselius apparatus (Kekwick, R. A. *Biochem. Journ.* 33: 1122, 1939). The application of Lamm's refractive index scale method permitted the determination of the relative concentrations of the various serum proteins with a fair degree of accuracy.

homologous type specific polysaccharide or of a heavy suspension of Type I pneumococci.

It has been possible to isolate certain antibodies from immune sera with the aid of the separation cell. Thus anti-egg albumin antibody has been obtained from rabbit serum by isolating the γ -globulin component which was specifically precipitable to an extent of about 80 per cent.

Examples for the electrophoretic differentiation of closely related proteins are provided by the formation of separate boundaries in mixtures of egg albumins or of hemoglobins of different species (Landsteiner, Longworth and Van der Scheer⁴⁴ in mixtures of fetal and adult hemoglobin of the same species (Adair³⁴); in mixtures of hemocyanins of different species (Tiselius and Horsfall⁴¹). Electrophoretic analysis has established the formation of hybrid hemocyanins upon reversible pH dissociation of mixtures of hemocyanins from different species and shifting of the pH back to the pH-stability range.

Other electrophoretic studies recently conducted in Upsala are concerned with the electrochemical properties of ovomucoid (Hesseltvik⁴⁵), with tuberculin proteins and polysaccharides obtained from the filtrates from culture media after growth of tubercle bacilli (Seibert, Pedersen and Tiselius⁴⁶) and with the homogeneity of pepsin preparations (Tiselius, Henschen and Svensson⁴⁷). In the latter investigation it was found that the enzymatic activity of solutions of crystalline pepsin, prepared by different methods, migrates as a homogeneous protein component with a negative charge and no isoelectric point in contrast to earlier observations made with the microcataphoretic method. Some inactive, inhomogeneous protein or protein breakdown material is left behind, and the specific activity may thereby be raised to an extent of from 31 to 69 per cent.

In conclusion, a few results obtained in our own work with the apparatus of Tiselius may be mentioned. A study has been made of crude extracts and purified fractions prepared from the anterior lobe of beef pituitary glands (Shipley, Stern and White Jour. Exp. Med. 69: 785. 1939) with a view of determining the number of protein components present in these preparations and of identifying certain of these components with the protein hormones elaborated by that gland. It was found that the main boundaries observable in crude pituitary ex-

⁴⁴ Landsteiner, K., Longworth, L. G., & Van der Scheer, J. Science 88: 83. 1938.

⁴⁵ Hesseltvik, L. Z. Physiol. Chem. 254: 144. 1938.

⁴⁶ Seibert, F. B., Pedersen, K. O., & Tiselius, A. Am. Rev. Tuberculosis 38: 399. 1938. Jour. Exp. Med 68: 413. 1938.

⁴⁷ Tiselius, A., Henschen, G. E., & Svensson, H. Biochem. Jour. 32: 1814. 1938.

tracts correspond to physiologically inert proteins the gradual removal of which by chemical fractionation procedures could be controlled by

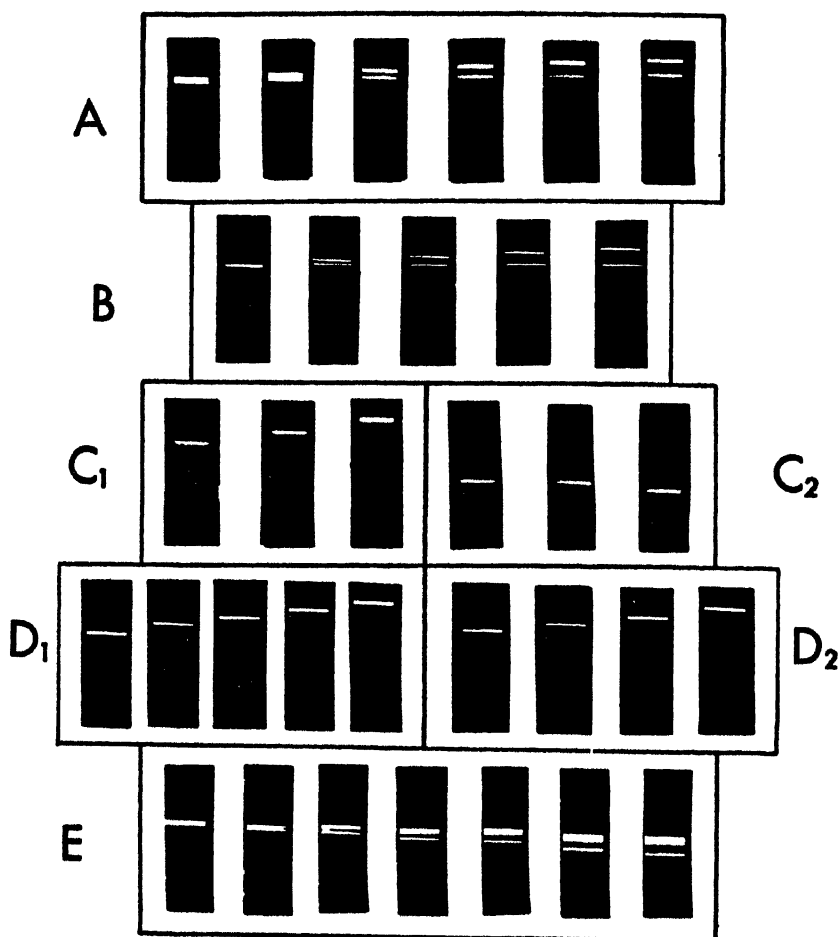


FIGURE 15. Toepler schlieren diagrams obtained upon electrophoresis of crude and purified anterior pituitary preparations. A. Crude Pituitary Extract (Burn & Ling) Anode chamber, pH 7.86. B. Crude Glycerol Extract, Anode Chamber, pH 7.65 (Boundary of slower protein retouched for purposes of better reproduction). C1. Growth-Ketogenic Fraction, Cathode Chamber, pH 4.86. C2. The same, Anode Chamber. D1. Crystalline Prolactin (Native) Cathode Chamber, pH 8.94, 1. Run. D2. The same, 2. Run. E. Crystalline Prolactin, Cathode Chamber, pH 6.5 Protein partly denatured.

electrophoretic analysis. Freshly prepared solutions of crystalline prolactin exhibit a high degree of electrochemical homogeneity which is lost upon storage. The formation of a second component in aged

prolactin solutions, probably of denatured prolactin, could be demonstrated by the appearance of a new boundary when examined by the schlieren method. The electrophoretic mobilities of the various components of pituitary preparations could be charted for the purpose of "labeling" them in order to gauge the success of fractionation procedures. FIGURE 15 contains characteristic schlieren diagrams obtained in this work.

Other experiments deal with the electrophoretic examination of chemically purified liver catalase solutions and of preparations obtained with the aid of the air-driven ultracentrifuge. Catalase solutions obtained by the method of Zeile and Hellstroem from horse liver yield up to four protein boundaries in the Tiselius apparatus. On the other hand, certain fractions obtained by differential ultracentrifugation by Wyckoff in collaboration with the writer not only proved to be homogeneous when examined in the analytical ultracentrifuge but they also gave only one boundary upon electrophoresis (FIGURE 16).⁴⁸

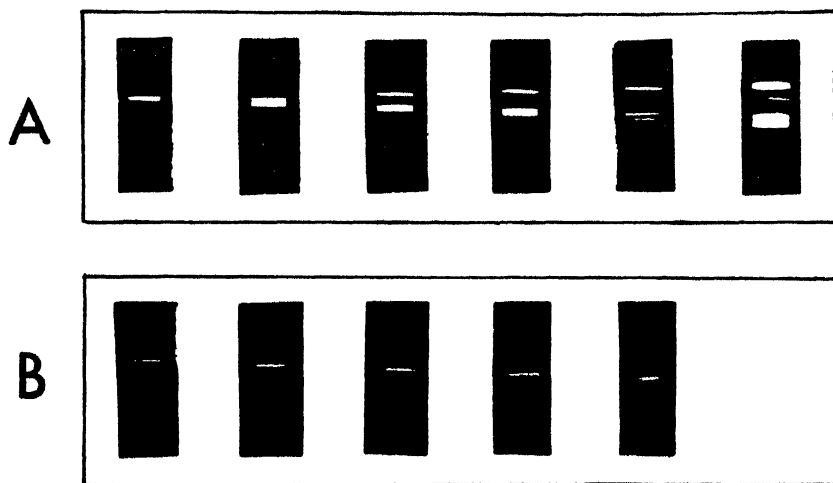


FIGURE 16. Electrophoretic diagrams obtained with chemically purified (A) and ultracentrifuged (B) preparations of horse liver catalase (Stern).

Such preparations which satisfy both the criteria of ultracentrifugal and of electrochemical homogeneity may be considered to represent solutions of the pure enzyme.

In the course of a study of heart muscle oxidase preparations (Stern,

⁴⁸ Stern, K. G. *Enzymologia* 5: 190. 1938.

Horwitt, Melnick, Scheff (unpublished)) the electrophoretic analysis of active fractions obtained by differential ultracentrifugation failed to effect separation into several different components indicating the surprisingly uniform nature of these active fractions. These preparations consist essentially of macromolecular protein particles of the enormous weight of several hundred millions. The spectroscopic and enzymatic activity tests reveal the presence of the following factors in these suspensions: Respiratory ferment, cytochrome *a* and *b*, and succinic dehydrogenase. These biocatalysts are essential components of the so-called "terminal" respiratory system which enables the molecular oxygen to react with the hydrogen of activated substrates. The ultracentrifugal and electrophoretic results obtained in the study of such oxidase preparations seem to suggest, as a working hypothesis, that they represent uniform suspensions of large protein bearer molecules or particles which contain on their surface a network of catalytically active groups arranged in such a manner as to ensure the smooth functioning of the complex mechanism of cell respiration.

THE OBSERVATION OF ELECTROPHORETIC BOUNDARIES

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INTRODUCTION

In the electrophoresis of proteins by the Tiselius method provision must be made for observing the progress of the migration. Since most protein solutions are colorless the position of the boundary between protein and buffer solutions is not visible to the unaided eye and optical devices must be employed. Three such devices have been used for the location and analysis of the concentration gradients which occur in both electrophoresis and ultracentrifugation. These are the absorption, the scale and the schlieren methods. These methods have been developed in the Upsala laboratories to a high degree of perfection by Svedberg,¹ Lamm,² and Tiselius.^{3, 4}

The absorption method depends upon the fact that proteins absorb light in the near ultra-violet while buffer solutions do not. The use of this method restricts the optical elements of the system to quartz and certain types of glass and is not applicable to solutes which do not absorb light in an accessible spectral region. Because of these and other limitations the author has not used the absorption method.

The application of the scale and schlieren methods, on the other hand, requires only that the refractive index of the solution differ from that of the solvent, a requirement that is almost universally met. Visible light may be used and, except for work of the highest precision, this does not need to be monochromatic if the lenses have sufficient chromatic correction. The author has begun a study and comparison of the two methods in connection with the electrophoresis of proteins and it is the purpose of this paper to describe the methods and discuss some of the results obtained with them.

¹ Svedberg, T. *Ind. & Eng. Chem. Analytical ed.* **10**: 113. 1938.

² Lamm, O. *Nova Acta Soc. Sci. Upsala* **IV**, **10** (6). 1937.

³ Tiselius, A. *Nova Acta Soc. Sci. Upsala* **IV**, **7** (4). 1930.

⁴ Tiselius, A. *Trans. Faraday Soc.* **33**: 524. 1937.

THE SCHLIEREN* METHOD

A diagram of the schlieren method is shown in FIGURE 1. An image of the horizontal slit, *S*, illuminated by the lamp, *L*, and condenser, *C*, is formed in the plane, *P*, by the schlieren lens *D*. The schlieren diaphragm, *A*, a screen with a sharp, horizontal upper edge, is placed in the plane *P* and may be displaced vertically with a micrometer. The electrophoresis cell, *E*, is placed as near the lens *D* as the thermostat construction permits. The camera objective, *O*, placed immediately behind the schlieren diaphragm, is focussed on the cell and forms a full size image of this on a ground glass or photographic plate at *G*.

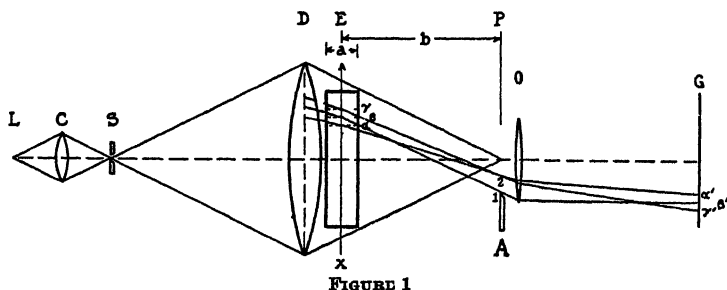


FIGURE 1

In the absence of refraction gradients, *i. e.*, boundaries, in the electrophoresis cell all of the light traversing the cell is brought to focus in the image of the illuminated slit at *P* and enters the camera objective. If, however, a boundary is present in the tube the refractive index, *n*, decreases with increasing height, *x*, through the boundary, and the pencils of light through this region are deflected downward. If these deflected pencils are intercepted by the schlieren diaphragm they do not enter the camera objective and the region at *G* conjugate to the boundary in the cell appears as a dark band on a light background.

For boundaries that are not too "sharp," *i. e.*, do not have too large refractive index gradients, the angular deviation of a horizontal pencil of light in a thin horizontal layer of the boundary is proportional (a) to the gradient, $\frac{dn}{dx}$, in the layer and (b) to the breadth, *a* (FIGURE 1), of the layer. The displacement, Δ , of the schlieren diaphragm, *A*, from the position of the undeviated slit image that is necessary to

* This term is defined in dictionaries of the English language as "streaks." Hardy and Perrin (Hardy, A. C., & Perrin, F. H. The principles of optics, McGraw-Hill Book Co., New York, 1932) use "shadow" as a synonym.

intercept the deflected pencil is also proportional to the optical distance, b , from the center of the cell to the diaphragm. Therefore

$$\Delta = ab \frac{dn}{dx} \quad (1)$$

in which a and b are constants of the apparatus and $\frac{dn}{dx}$ varies vertically through the boundary but is assumed to be constant in any thin horizontal section.

As the schlieren diaphragm is raised the first pencils of light to be intercepted are those which have passed through the steepest gradients of refractive index. In FIGURE 1 the paths of three pencils of light through a boundary have been traced. The planes α , β and γ in the boundary have been selected such that

$$\left(\frac{dn}{dx}\right)_{\alpha} = \left(\frac{dn}{dx}\right)_{\gamma} < \left(\frac{dn}{dx}\right)_{\beta} = \left(\frac{dn}{dx}\right)_{max.}$$

and the lines conjugate to these in the focal plane of the camera are shown at α' , β' and γ' , respectively. Since the camera objective, O , is focussed on the electrophoresis cell E all of the rays passing through the plane β , for example, that are collected by this objective are brought to focus at β' . This, it will be recalled, is the property of a photographic objective. Moreover, it should be noted that in FIGURE 1 the vertical dimensions are greatly exaggerated in comparison with the horizontal dimensions.

The pencil through β , FIGURE 1, suffers the maximum deflection to the position 1 near the plane of the schlieren diaphragm while those through α and γ are deflected equally to a common position 2 near that plane. With the upper edge of the diaphragm at 1 only the pencil through β is intercepted and the resultant dark band, or line, at β'

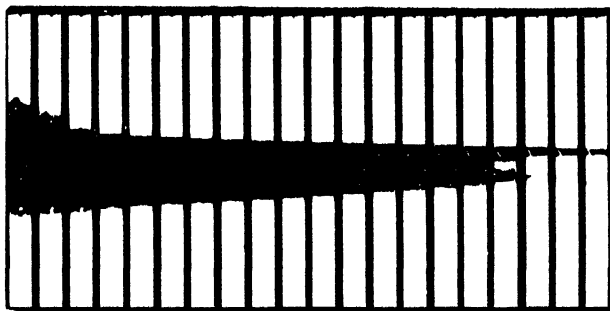


FIGURE 2

has a minimum width. With the diaphragm raised to position 2 the pencils through α and γ , together with the pencils through all planes between α and γ , are also intercepted and the band at G has broadened correspondingly. With the proper conditions the displacement of the diaphragm from the position of the undeviated slit image is proportional to the refraction gradient at positions in the cell E conjugate to the edges of the schlieren bands. Thus a series of photographs of the boundary with decreasing displacement of the diaphragm give an

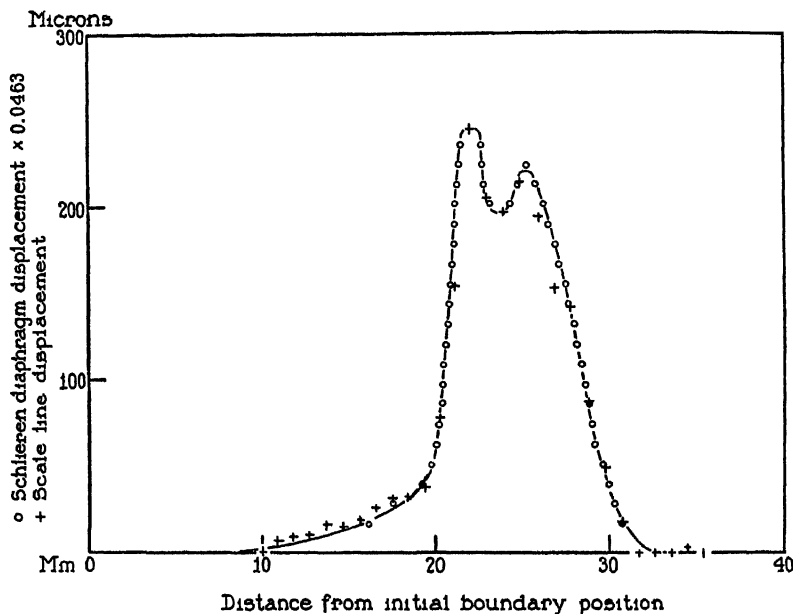


FIGURE 3

indication of the refraction gradient through the boundary. Such a series of photographs of the boundary formed in a 0.5 per cent solution of a sample of egg albumin is shown in FIGURE 2. In order for the position of the edge of a band to be characteristic of a thin horizontal section of the refraction gradient it is essential that lenses of long focal length be used and that the slit image be narrow vertically and well defined. Consequently the schlieren lens must be well corrected spherically, and also chromatically, unless monochromatic light is used.

In FIGURE 3 the circles indicate the positions of the edges of the bands of FIGURE 2, plotted as abscissae against the diaphragm displacements, multiplied by a factor whose significance will be discussed

later in this paper, as ordinates. The origin of abscissae was taken as the point of initial formation of the boundary, *i. e.*, between the horizontal plates of the center sections of the cell. The origin of ordinates was taken as the center of the undeviated slit image. A comparison of these results with those obtained with the scale method is made later in this paper.

Reference to equation (1) shows that the area under the curve drawn through the circles is

$$\int \Delta dx = \int abd\eta = ab\Delta\eta$$

in which $\Delta\eta$ is the refractive index increment due to the protein causing the boundary. Since this increment is substantially proportional to the concentration of protein it becomes apparent that the schlieren method may be used to determine the protein concentration in addition to the variation of this through the boundary. Thus the area under the curve of FIGURE 3, as determined with the aid of a planimeter, is 0.414 cm^{-2} . Since $a = 2.50 \text{ cm}$ and $b = 181.8 \text{ cm}$, $\Delta\eta$ (obsd.) = 0.00091 . An independent value for $\Delta\eta$ of 0.00092 was computed as follows. The refractive index increment⁵ for a 0.5 per cent solution of ovalbumin at 23° using the sodium *D* line is 0.00088_6 . Assuming that the temperature coefficient for the increment is zero but that the dispersion is the same as for serum albumin⁶ (Pederson & Anderson) the value of $\Delta\eta$ given above was computed for the Hg 4356 line, to which the plate used had the greatest sensitivity. The agreement between the observed and computed values is thus quite satisfactory.

DIFFRACTION BY THE SCHLIEREN DIAPHRAGM

Since it is desirable that the edge of a schlieren band be sharply defined the factors which determine this sharpness will be considered here. These factors are (1) the characteristics of the photographic plate, (2) the vertical width of the undeviated slit image in the plane of the schlieren diaphragm, and (3) the nature of the diffraction pattern formed at the plate by the diaphragm. These will be discussed in the order given.

Since, as will become evident from the paragraphs to follow, there is always some shading at the edge of a schlieren band a plate of high contrast and minimum latitude gives the steepest gradient of optical density at the band's edge.

⁵ International Critical Tables 7: 93. McGraw-Hill Book Co., New York. 1930.

⁶ McFarlane, A. S. Biochem. Jour. 29: 412. 1935.

If the vertical width of the undeviated slit image is finite, it is evident from FIGURE 1 that there is a shading at the edge of the schlieren band due to the fact that the diaphragm does not intercept all of the pencils of light deflected by the layer in the cell conjugate to the edge of the band. A *very* narrow slit image, even if it could be realized in practice, is of little value, however, since some shading at the edge of the schlieren band still occurs due to diffraction by the diaphragm and the finite latitude of the photographic plate. In obtaining the bands of FIGURE 2 the width of both the slit and its image was about 0.1 mm. Slit widths of 0.2 and 0.05 mm have also been used with no discernible difference in the sharpness of the resulting band edges. In this connection it may be mentioned that, as the other extreme, if the width of the undeviated slit image is greater than the maximum deviation at the diaphragm, the latter may be placed in such a position that the shading at the plate is proportional to the deviation. This is the modification of the schlieren method discussed by Schardin.⁷

Owing to the long optical paths that are used the diffraction patterns formed at the photographic plate by the schlieren diaphragm are sufficiently gross to be readily visible in many instances. In the case of a straight edge as diaphragm the pattern consists⁸ of "a system of fringes of decreasing width, outside of the edge of the geometrical shadow, while within the edge the illumination falls off rapidly, without, however, passing through maxima and minima." Although the decrease in illumination within the edge of the shadow is rapid it is not abrupt, as implied in the theory of the rectilinear propagation of light, with the result that there must always be some shading at the edge due to diffraction alone. The fringes mentioned above appear as faint bands in the illuminated regions on either side of a schlieren band and can be readily distinguished from the latter by the fact that their position shifts with the shifting edge of the true band as the diaphragm is raised or lowered.

In his study of the schlieren method Lamm used as light source an illuminated slit of 0.2 mm width and, as schlieren diaphragm, single, and double, slits of 0.5 mm width and wider. Reference to FIGURE 1 indicates that the use of a slit instead of a straight edge as schlieren diaphragm produces on the ground glass lines of light at positions corresponding to the edges of the schlieren bands. The optimum dia-

⁷ Schardin, H. Das Toeplersche Schlierenverfahren. VDI-Verlag, G. M. B. H., Berlin, 1934.

⁸ Wood, E. W. Physical Optics, McMillan Co., New York, 1924.

phragm slit width appears to be that which covers the central "Fresnel zone."⁸ For lenses of 100 cm focal length and blue light this corresponds to a slit width of 1 mm and the lines formed on the screen are correspondingly broad.

A comparison of the diffraction patterns formed by a slit and a straight edge may help in discussing the question of which of these is the most desirable diaphragm. The patterns formed by a slit are symmetrical about the center, whereas, as has been mentioned, a straight edge has no diffraction pattern in the region of the geometrical shadow. This difference is both the weakness and the strength of the straight edge as schlieren diaphragm. Its weakness lies in the fact that, whereas the position of the center of the line formed on a photographic plate by a slit is independent of the exposure, the position of the edge of a band obtained with a straight edge depends slightly on that exposure. Its strength lies in the fact that these edges are very much sharper than the lines obtained with a slit diaphragm, with the result that the resolving power of the straight edge for boundaries that are close together is greater.

THE OPTICAL SYSTEM

Since it is the spirit of this conference to discuss technique and apparatus as well as results I will describe our schlieren equipment. A diagram of the optical bench, including the low temperature thermostat, is shown in FIGURE 4. The letters in this figure indicate the same optical elements as in FIGURE 1. The entire apparatus is supported on concrete blocks, *B*, which rest on shock absorbing mats placed on a ground floor. The optical bench consists of two 5" steel channels, 21' in length, as shown at *C* in FIGURE 4. These are spaced 8" apart at 4' intervals with sections of iron pipe as shown at *B* in FIGURE 5. This latter figure also illustrates the method of mounting the optical elements. A 6" section of a 6" x 3/8" steel angle, *A*, is clamped to the upper flanges of the channels, *C*, with the aid of the plate, *P'*, and the bolts *b*. The lens or other optical element is carried by a 6" x 16" x 3/8" steel plate, *P*, which is bolted to the angle through slots as shown at *a*. This arrangement, utilizing standard structural steel shapes, provides for flexibility of adjustment.

The lamp *L*, FIGURES 1 and 4, is a 100 watt mercury arc, General Electric Vapour Lamp Co. type H4. The dimensions of the arc are about 1.5 x 25 mm and it has a high intrinsic brilliance. Operated in a horizontal position it is an excellent source of illumination for the

slit at *S*. The dimensions of the slit are adjustable. The length generally used is 25 mm while the width, determined with the aid of a "feeler" gauge, has been varied from 0.05 to 0.5 mm. The condenser *C* is a 40 mm achromat of 100 mm focal length. Used at unit magnification it is able to fill the lens *D* uniformly with light.*

The schlieren lens *D* is a 4" achromat of 36" focal length prepared by Perkin, Elmer and Moffitt of this city. It has the same corrections as a telescope objective except that it is for use at unit magnification. The chromatic correction is for photographic work, *i. e.*, green and blue. Most of the energy of the light source is in these wavelengths and, if it were not for the chromatic aberrations introduced by the boundaries, the use of monochromatic light would not be necessary.

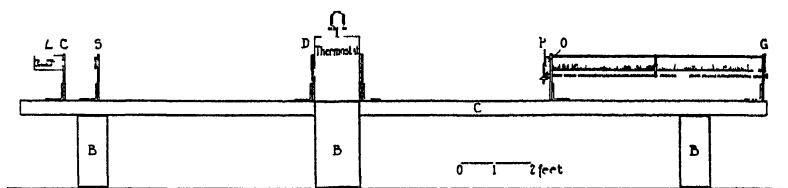


FIGURE 4

However, due to these aberrations the use of a narrow band of wavelengths increases the sharpness of the schlieren band edges somewhat. A Wratten mercury monochromat filter Number 50, for isolation of the Hg 4356 line, has been used in some recent work.

The schlieren lens is shown at *D* in FIGURE 6 as one of the thermostat windows. This figure is a cross section of the low temperature thermostat and electrophoresis cell. The construction of the double windows shown is necessary in order to prevent condensation of moisture. With the exception of the schlieren lens the windows, *W*, are $4\frac{1}{2}$ " discs of $\frac{1}{4}$ " plate glass. These were selected for freedom from flaws and then ground flat to within a few wavelengths of light. The discs, *W*₂ and *W*₃, are clamped against rubber gaskets supported by the bakelite ring, *a*, and the space between is filled with dry air and sealed. The space between the schlieren lens and *W*₁ could not be made completely air tight. Consequently provision was made to pass dry air

* It is also possible to illuminate a slit of these dimensions directly with the H₄ lamp since the size of the latter is such that, with the lamp placed immediately behind the slit, the angle subtended by the arc at the slit is greater than that subtended by the schlieren lens. This arrangement, suggested by Dr. E. G. Pickels of the International Health Division of the Rockefeller Foundation, has proved more desirable than that described above since the condensing lens is thereby eliminated and the optical bench shortened by some 40 cm, with no decrease in light intensity.

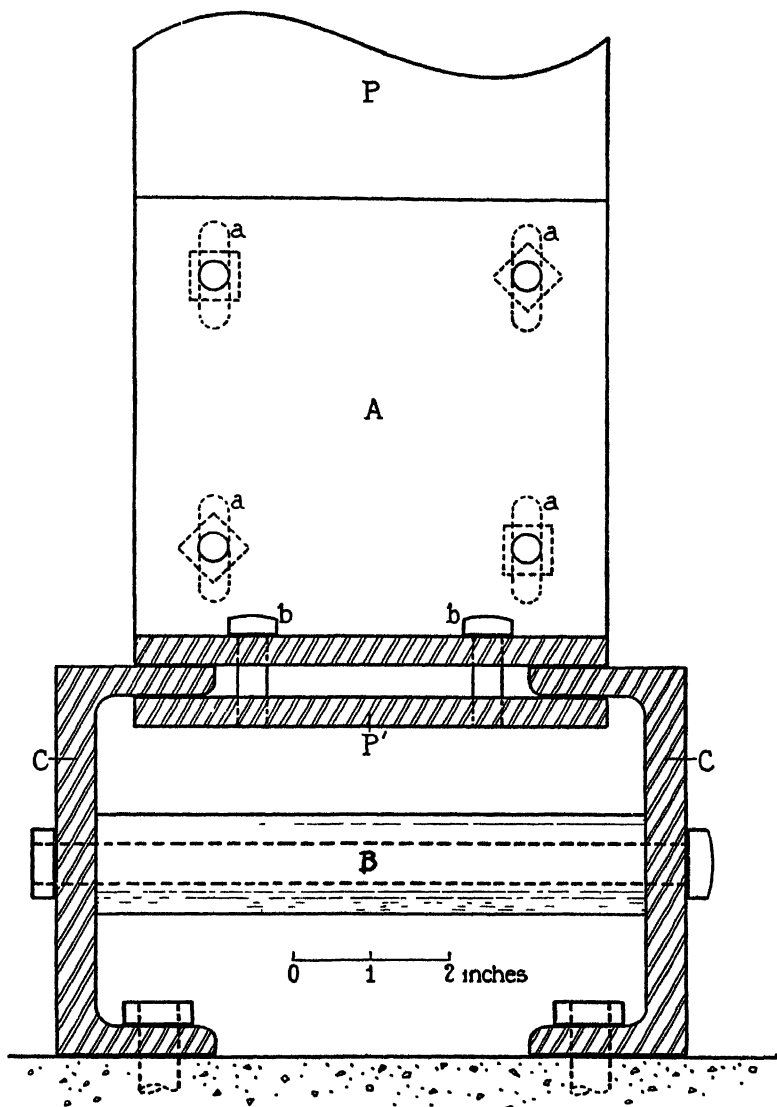


FIGURE 5

through this space when necessary. The schlieren lens and thermostat windows are kept in alignment by means of the plates, *P*, attached to the optical bench.

The schlieren diaphragm and camera are shown in profile in FIGURE 7. The diaphragm, *d*, is a brass plate with a sharp horizontal upper

edge. It is carried by two rods, r , which slide in the tubes, t , and may be moved with the micrometer, m , which reads directly to 0.01 mm. The bevel gears, b , and shaft, s , permit adjustment of the diaphragm when viewing the ground glass screen at G .

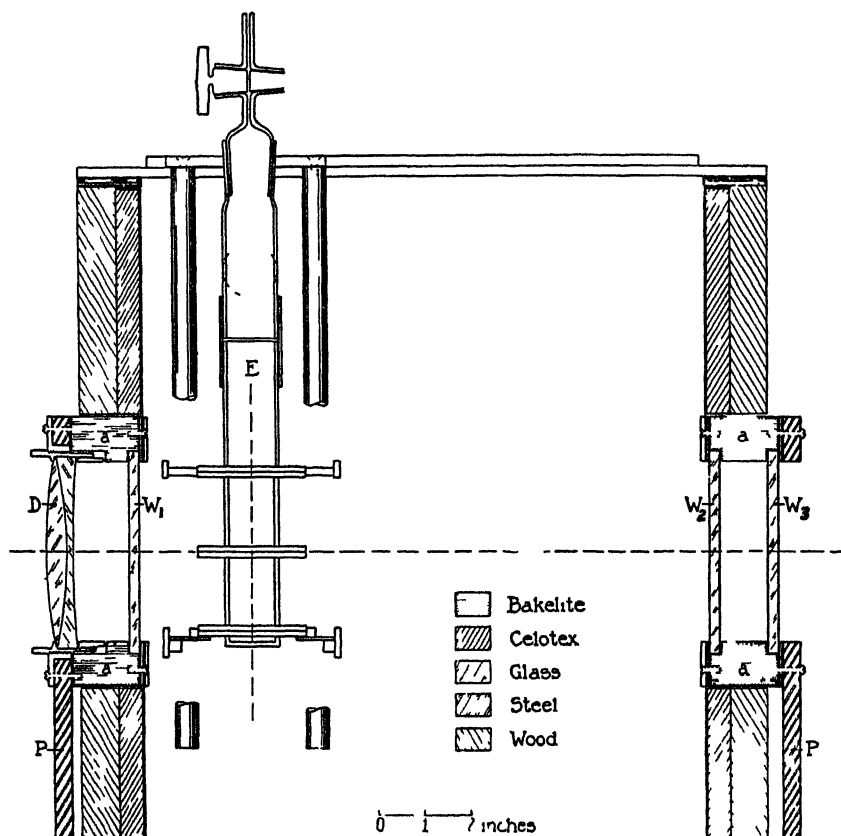


FIGURE 6

The camera objective O , FIGURE 7, is a 2" achromat of 36" focal length and has the same corrections as the schlieren lens. Provision for adjustment of this lens along the optic axis has been made at A . This adjustment has proved useful in obtaining unit magnification. A displacement of the lens O of 1 cm alters the enlargement factor by 1 per cent but shifts the image plane by only 1 mm. Precise control of this factor is desirable in using the scale method since it is convenient to have the interval of the undeviated scale image a simple multiple of the comparator thread.

The lens *O* is also provided with a sector type shutter, *c*, driven by a synchronous motor, *e*, and a series of aperture stops, *a*, the smaller ones having the shape shown in FIGURE 8. A stop of this shape is preferable to a circular one since it permits use of the full lens aperture in the direction in which the pencils of light are deflected, thus insuring that the lens stop will not act as schlieren diaphragm. As a matter of

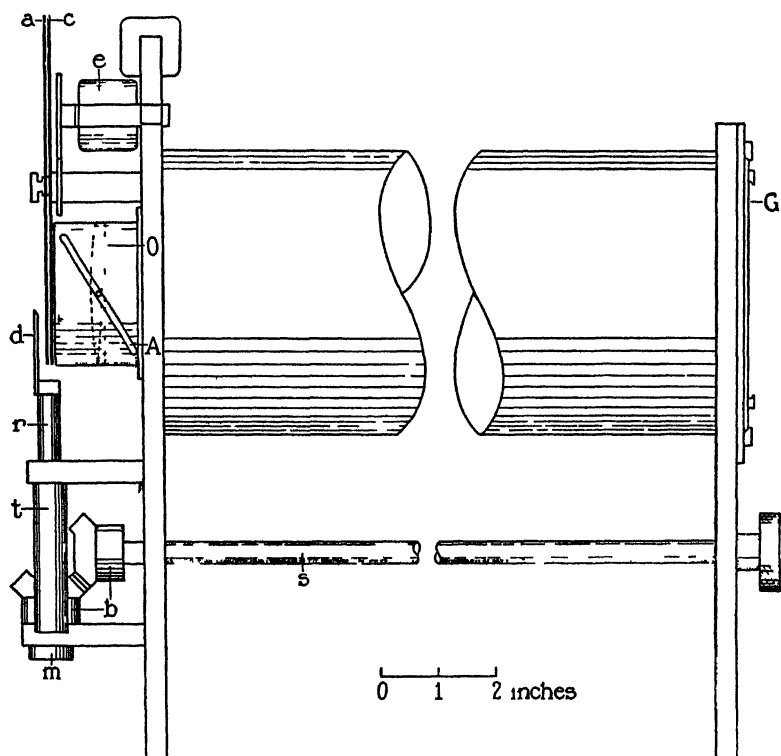


FIGURE 7

fact this 2" objective is not large enough to collect the pencils deviated by steep gradients, in which case the lens barrel acts as a schlieren diaphragm.

The plate holder at *G*, FIGURE 7, is provided with appropriate masks so that several exposures can be made on one plate. Thus if all of the protein constituents are migrating as anions, for example, there are no boundaries in the lower anode and upper cathode sections and these can be masked. Twenty-two exposures of the sections in which boundaries appear can then be made on a 9 x 12 cm plate, as was

illustrated in **FIGURE 2**. Except when red sensitive plates are necessary, as in work with hemoglobin for example, we have used Eastman contrast lantern slides. The slow speed, high contrast, fine grain and

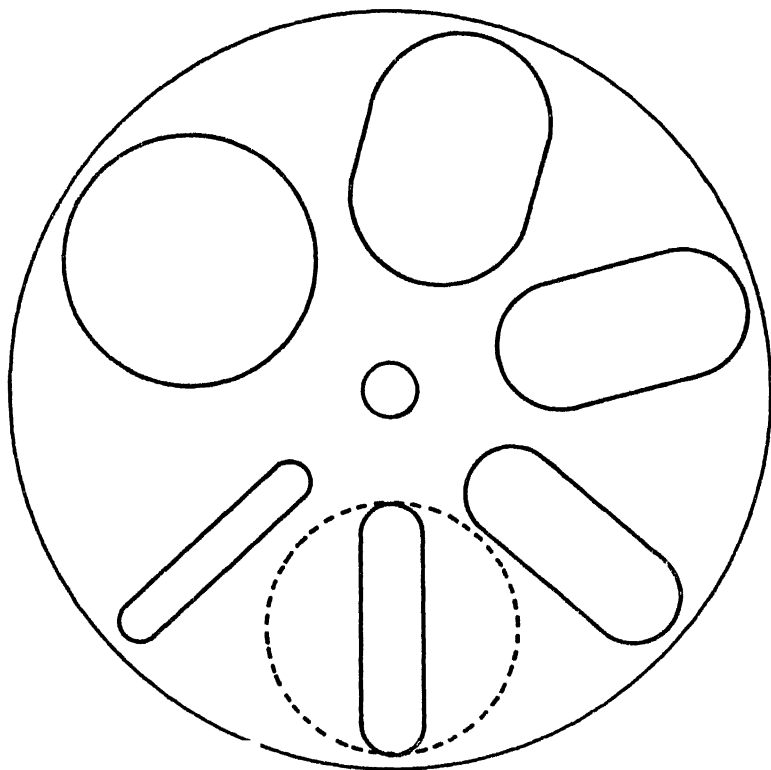


FIGURE 8

case of manipulation of these plates make them well adapted for use with both the schlieren and scale methods. Using schlieren observation of hemoglobin boundaries it was necessary to mask the color of this protein. This was done with a Wratten A filter and Wratten M plates were used in photographing the boundaries.

THE SCALE METHOD

Very minor changes in the optical system described in the preceding section make it possible to use the scale and schlieren methods interchangeably. With the latter method the center of the electrophoresis cell, *E*, **FIGURE 6**, is in the focal plane of the camera. In using the

scale method the cell is moved toward the camera a few centimeters and a transparent scale, having two lines per millimeter for example, is placed in the position previously occupied by the cell. The scale is then photographed through the cell with the schlieren diaphragm removed so that schlieren bands will not obscure certain of the scale lines. The schlieren lens in this case acts merely as a projection condenser.

The diagram of FIGURE 9 will serve to illustrate the theory of the scale method. In the absence of refraction gradients in the cell, E , a scale line, s , is brought to focus at s' by the lens O . If, however, there is a gradient in the cell between the planes pp' the rays from s which are collected by the lens have been deflected downward as shown in

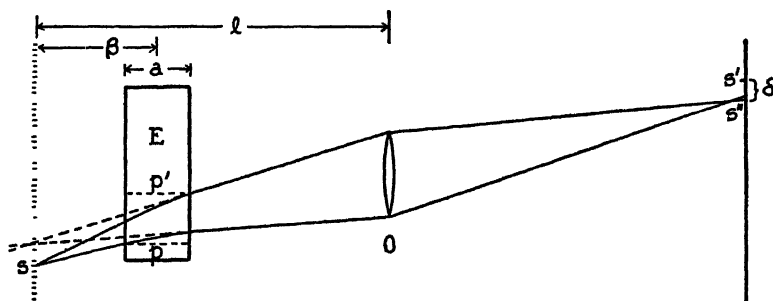


FIGURE 9

the figure and intersect approximately at s'' . Due to the faulty lens action of the gradient the image of s at s'' is imperfect. With sufficient depth of focus s'' appears on the plate as a line displaced by an amount δ from its position s' in the absence of gradients in the cell. In order for the displacement of each line to be determined by the gradient in a thin horizontal section of the column the lens O should have a long focal length and be used at a small aperture. The latter condition is also desirable in order to increase the focal depth.

In practice the scale is photographed through the electrophoresis cell before the boundaries have appeared from behind the horizontal glass plates and then again at some later time after they have migrated into view. The positions of corresponding lines on the two photographs may then be determined with the aid of a comparator and the observed displacements, δ , of the lines of the second photograph in the neighborhood of the boundary computed.

Again assuming that the gradients are not too steep, the displacement, δ , of a scale line is proportional to (a) the horizontal breadth, a ,

of the boundary, (b) the *optical* distance, β , from the scale to the center of the cell and (c) the gradient, $\frac{dn}{dx}$. For unit magnification, therefore,

$$\delta = a \beta \frac{dn}{dx} \quad (2)$$

Since the electrophoresis cell undergoes an apparent enlargement when moved out of the focal plane toward the camera, the position in the gradient causing a given displacement, δ , is given by the corresponding value of s'' reduced by the factor, $\frac{l - \beta}{l}$, in which l is the optical distance from the scale to the camera. The scale photographs of the boundaries shown in FIGURE 2 were obtained with a scale distance $\beta = \frac{11.2}{1.33} = 8.42$ cm. Since $l = 184.8$ cm, the reduction factor is 0.9545. In FIGURE 3 the crosses indicate the scale line displacements, in microns, plotted against the displaced line positions multiplied by the factor 0.9545. A comparison of equations (1) and (2) indicates that, for a given gradient and cell thickness, $\frac{\delta}{\Delta} = \frac{\beta}{b}$, i. e., the scale line and schlieren diaphragm displacements are in the same ratio as the "optical lever arm lengths" β and b . The schlieren photographs of FIGURE 2 were obtained with $b = 181.8$ cm. Consequently $\frac{\beta}{b} = 0.0463$ and it was this factor by which the schlieren diaphragm displacements were multiplied in plotting the circles of FIGURE 3. When plotted in this manner the scale line and schlieren diaphragm displacements fall on the same curve. This agreement between the two methods, together with the agreement between the computed and observed values of Δn previously noted, is evidence for the validity of the simple theory considered in this paper.

DISCUSSION OF THE SCALE AND SCHLIEREN METHODS

From the results given in this paper it appears probable that the precision attainable with the schlieren method is comparable with that of the scale method. The latter method is the more economical of the two, however, since the schlieren lens is unnecessary, only the relatively small and inexpensive camera objective being required. More-

over, it is the more flexible of the two methods since it is much more practical to change the scale distance, β , than the diaphragm distance, b . However, the comparing of the scale line photographs is a laborious operation which does not lend itself to mechanization. Also the results of the experiment are not known until this operation has been completed.

With the schlieren method, on the other hand, the progress of the boundaries can be followed visually from the beginning of the experiment. By observing the initial appearance of the bands on the ground glass and their broadening as one raises the schlieren diaphragm an idea of the homogeneity and relative amounts of each component can be obtained immediately. Moreover, if one is measuring mobilities only and can assume the region of steepest gradient to be the center of the boundary, the schlieren band positions can be read directly from a ruling on the ground glass. Although a quantitative analysis of sharp boundaries is not possible with either method the schlieren arrangement has a greater resolving power for boundaries of this type that may be close together, as in the early stages of serum electrophoresis.

The schlieren method also lends itself readily to a direct photographic or photo-mechanical recording of the refraction gradients through the boundary. As was mentioned earlier in this paper, Schardin has shown how the light source may be arranged to give a blackening of the photographic plate that is proportional to the gradient. We have avoided this arrangement, however, since, in common with the absorption method mentioned in the introduction, the plate density would have to be determined with a micro-photometer. Philpot⁹ has described a novel arrangement of slit and diaphragm which produces automatically on the plate a light area whose contour is similar to the curve of FIGURE 3. The author is now testing the following arrangement which has a similar purpose but appears to be more flexible than Philpot's device. The cell image is masked by a narrow vertical slit. A glance at FIGURE 2 then suggests that if the plate be driven slowly in a horizontal direction simultaneously with the vertical displacement of the diaphragm a light area is produced automatically on the plate whose contour is identical with the curve of FIGURE 3. The scale of ordinates for this area can be adjusted by controlling the relative rates of movement of the plate and diaphragm. Moreover it should be possible to determine the magnitude of the light area on the plate photometrically, as Philpot suggests, or directly

⁹ Philpot, J. S. L. *Nature* 141: 283, 1938.

with a planimeter. It will be of interest to see what precision can be attained with this method.*

The author takes pleasure in acknowledging his indebtedness to Dr. D. A. MacInnes of these Laboratories for his continued support and sustained interest in the work reported in this paper.

* Since this paper was presented on October 29, 1938, the author has had the opportunity of testing the modification of the schlieren method mentioned above. The results have been very encouraging and have been reported briefly elsewhere (Longworth, *L. G. Jour. Am. Chem. Soc.* **61**: 529. 1939).

CHARACTERISTICS OF PROTEIN BOUNDARIES AS SHOWN BY SCALE METHOD ELECTROPHORETIC DIAGRAMS

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The experiments and observations upon which this discussion is based were carried out under the direction and supervision of Professor Arne Tiselius at the Physical Chemistry Institute in Upsala, Sweden. All of the results which are presented here in summary form have been published in detail elsewhere by Tiselius and Horsfall.¹

In order to study with precision the characteristics of moving boundaries of protein in electrophoresis, a well defined test object was necessary. Various hemocyanins were selected for this purpose because certain of these proteins are fairly well characterized when studied chemically, in the ultracentrifuge, by diffusion, and in electrophoresis. They also possess the advantages attributable to their very high molecular weights and low diffusion constants.

All experiments were carried out in the improved electrophoresis apparatus described by Tiselius.² The scale method of Lamm³ which has recently been adapted to electrophoresis was used throughout for the quantitative study of protein boundary phenomena. Multiple scale method electrophoretic diagrams were worked out in each experiment so that the whole course of the migration of the boundaries in both limbs of the U tube could be followed accurately.

Although the micro-comparison of the position of scale lines, the plotting of scale line displacement graphs, and the integration of areas beneath the curves obtained is laborious and time consuming, the accuracy of the method and the additional information gained often well repay energy so expended. Scale method electrophoretic diagrams may reveal minor degrees of boundary inhomogeneity which would be difficult to assess accurately by other methods. They also permit the identification and the calculation of mobilities for minor components which fail, even after maximum migration, to resolve into distinct boundaries separate from those of the major components. And finally scale method diagrams make it possible to calculate the

¹ Tiselius, A., & Horsfall, F., Jr. *Nova Acta Soc. Sci. Upsala* (in press). 1939.

² Tiselius, A. *Trans. Faraday Soc.* **33**: 524. 1937.

³ Lamm, O. *Z. Physik. Chem.* **A138**: 313. 1928.

concentration of separate components even though the quantity of protein contained in them is relatively small. Component concentrations determined optically by this method have been found to be as accurate as micro-Kjeldahl analyses of the same solutions.

Certain unnecessary anomalies in both boundary form and migration may easily confuse the interpretation of electrophoretic studies. Protein solutions which have not been dialysed against buffer for a sufficiently long period usually produce abnormal boundaries which may migrate with different mobilities in the two limbs of the U tube, may appear unusually sharp in one limb, and even may produce more than one concentration gradient peak. Protein solutions which have been adequately dialysed but against a buffer of insufficient ionic strength may also migrate in an abnormal manner, and frequently under these conditions only a fraction of the total protein present in the U tube will enter into the formation of the moving boundary. Even though dialysis has been prolonged sufficiently to achieve equilibrium between the protein and the buffer, and despite the use of a buffer of adequate ionic strength, the concentration of protein itself is of importance and has definite upper limits in quantitative studies. With too great concentrations of protein the so-called δ boundary may develop. Most accurate results, both as regards mobility determinations and component concentration calculations, are achieved when relatively dilute protein solutions are used. Heat convections may arise if the potential gradient exceeds definite limits or if the effective removal of heat from the U tube itself is inefficient. One of the first indications of the development of convections is an upward convexity of one or both boundaries as observed by the Toepler Schlieren method. With reasonable care and with a properly constructed and operated apparatus it is possible to prevent the occurrence of any of the boundary anomalies which have been mentioned above.

The study of multiple scale method diagrams of the moving boundaries of hemocyanins has revealed a slight and constant asymmetry of both the ascending and the descending boundaries. This asymmetry is usually apparent when the scale line deflection curves are plotted, but it is more readily recognized when the two areas under the curve and on either side of a vertical line through the point of maximum deviation are integrated and compared. The ascending boundary (A) and the descending boundary (D) shown in FIGURE 1 illustrate this asymmetry.

It is to be noted that in the case of both boundaries the area (B) between the vertical line and the buffer is smaller than the area (P)

between the vertical line and gradient free protein. With hemocyanins at least, this bilateral boundary asymmetry seems to be a constant phenomenon and occurs whether the boundary is moving into buffer or into protein. Although this slight asymmetry in boundary form does occur, it does not complicate the determination of mobilities or concentrations. The asymmetry is thought to be caused by minor heat convections, and it is unlikely that even under the most ideal conditions these can be entirely eliminated.

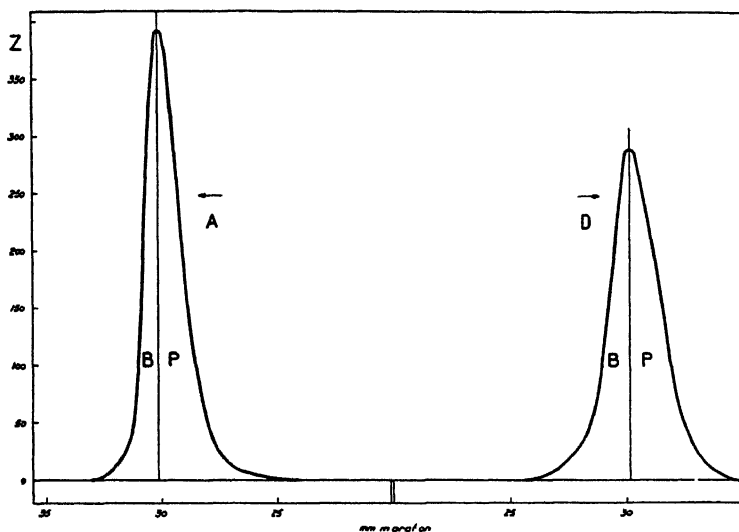


FIGURE 1

Scale method diagrams have also demonstrated that the ascending boundary is regularly somewhat more sharp and slightly less broad than the descending boundary. That is, the maximum refraction produced by the ascending boundary is greater than the maximum refraction produced by the descending boundary, and the vertical distance in the U tube through which the ascending boundary causes refraction is less than the corresponding distance for the descending boundary. The two diagrams shown in FIGURE 1 also illustrate these characteristics. The areas under the curves for the two boundaries are, however, identical within the limits of experimental error, and the protein concentration can readily be calculated from either.

The various quantitative characteristics of ascending and descending hemocyanin boundaries which have been briefly referred to above may be observed at any one period during a single experiment and are

not dependent upon either time or the distance of migration. Comparisons of multiple scale method diagrams, however, taken at different times during one and the same electrophoretic experiment show still another and a much more striking phenomenon, that is boundary spreading. This phenomenon was observed by Tiselius,⁴ and it was shown that its occurrence precluded the calculation of diffusion con-

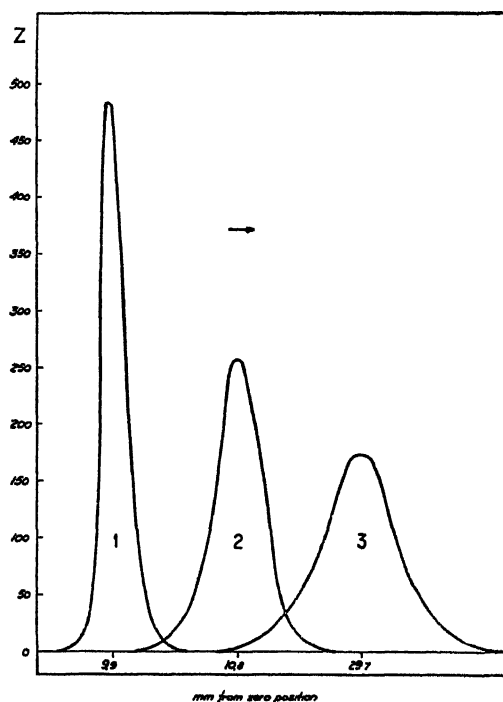


FIGURE 2

stants during electrophoresis. Both the ascending and the descending boundaries become progressively less sharp and more broad as they migrate farther from zero position. This spreading, which is associated with increasing migration, affects both boundaries in equal proportions and can readily be shown to be neither the result of diffusion nor due to an apparatus effect by simply reversing the current. When this is done not only is the direction of migration of the boundaries reversed but so too is their spreading, and as the boundaries migrate back towards zero position they become progressively more sharp and less

⁴ Tiselius, A. *Nova Acta Soc. Sci. Upsala* IV, 7 (4), 1930.

broad. In FIGURE 2 are shown three scale method electrophoretic diagrams of a single hemocyanin boundary as it migrated away from zero position. In FIGURE 3 are shown three diagrams of this same boundary as it migrated back towards zero position after reversal of the current.

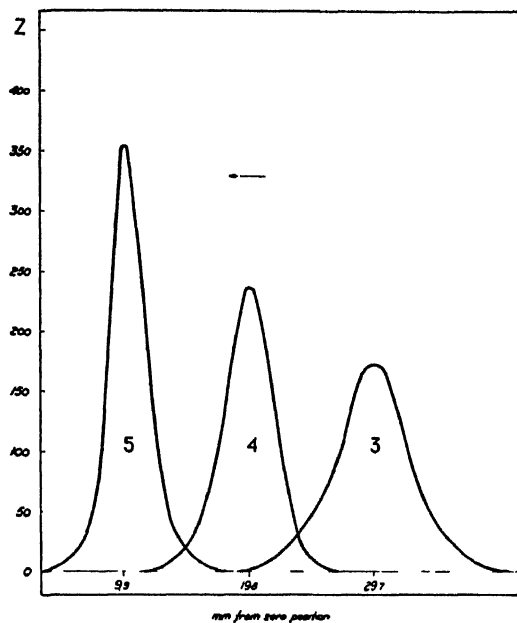


FIGURE 3

Boundaries which have migrated as far as possible within the cell and have then been reversed and have migrated back to zero position do not completely regain their original sharpness, although they may approximate this state rather closely. This phenomenon has been termed reversible boundary spreading and has been interpreted as evidence of some electro-chemical inhomogeneity in the protein producing the boundary.

THE INFLUENCE OF IONIC STRENGTH AND pH ON ELECTROPHORETIC MOBILITY

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The method of electrophoresis was first employed in the study of a protein solution when Picton and Linder¹ noted in 1892 the movement of hemoglobin in an electric field. In the same decade W. B. Hardy^{2, 3} extended the use of the method to other proteins and to other solvents, and noted 1) that "the particles, since they refract light, must be of much more than molecular dimensions" and 2) that the charge was "due to a real electrolytic dissociation at the surface of the particles, the degree of dissociation depends upon the same factors, which in the interaction between solute and solvent determine the degree of dissociation of different electrolytes"³ (p. 292).

Hardy also noted that proteins "move with the negative stream if the reaction of the fluid is alkaline; with the positive stream if the fluid is acid"² (p. 304), and thus demonstrated their amphoteric nature. Michaelis⁴ was however the first investigator to determine accurately the hydrogen ion concentration at which the migration of a protein changed its direction, termed the isoelectric point, and to apply dissociation theory to characterize the isoelectric point of a simple ampholyte.

Proteins are multivalent both as acids and as bases and exist largely as dipolar ions in the neighborhood of their isoelectric point. At other reactions they possess a net charge and are present as salts. The valence of the protein thus might be expected to influence mobility in two ways. Thus the greater the valence the greater the net charge and therefore the greater the mobility. On the other hand, the greater the valence the more the activity coefficients deviate from unity at any given concentration and the more the mobility will diminish from that which would obtain in infinitely dilute solution. Mobility should be closely proportional to the net charge under the latter conditions, but not necessarily at appreciable ionic strengths.

There have been numerous improvements in the technique of measuring electrophoretic mobility since the early measurements of

¹ Picton, H., & Linder, S. E. *Jour. Chem. Soc.* 61: 148. 1892; 71: 568. 1897.

² Hardy, W. B. *Jour. Physiol.* 24: 158, 288. 1899.

³ Hardy, W. B. *Jour. Physiol.* 33: 251. 1905.

⁴ Michaelis, L. *Die Wasserstoffionenkonzentration*. Berlin. 1914, 1922.

Picton and Linder¹ and of Hardy.^{2, 3} Omitting from consideration the microscopic methods which have been extensively employed,^{5, 6, 7} these include protecting the protein solution from changes in hydrogen ion concentration occurring at the electrodes, determining conditions such that the change in density, in conductivity and in the gradient of temperature and pH and other factors at the moving boundary be reduced to a minimum. Despite the great advances that have been made, especially by Tiselius, mobility is still determined in the presence of a relatively high concentration of other ions, generally of buffer ions, and it is therefore desirable to consider not only the influence of the pH but of the ionic strength of the solution.

The movement of the hemoglobin boundary is readily followed without the aid of an optical system such as that employed by Scott and Svedberg⁸ and later by Tiselius.⁹ Hemoglobin, investigated by Michaelis and Airila in 1921,¹⁰ has therefore repeatedly been studied in our laboratory since the early measurements of Laszlo Reiner.¹¹ Recent studies have moreover demonstrated that the dissociation of horse carboxyhemoglobin over the neutral range is due to the imidazole groups of histidine. Of the 32 or 33 such groups per molecule of hemoglobin from 12 to 16 appear to dissociate at reactions acid to the isoelectric point but alkaline to pH 5.6.¹² Studies of the mobility of the protein under these conditions were therefore undertaken in order to determine the relative importance of pH and ionic strength when there was some knowledge of the number and nature of the dissociating groups. The most recent form of the Tiselius apparatus, which should make possible comparable studies upon other proteins, was not available when these measurements were begun, but has since become so and the results with the simpler electrophoresis apparatus employed¹³ have been confirmed with it.

At an ionic strength of 0.02 and a pH of 5.65 a mobility of 10.3×10^{-5} cm²/volt-sec was observed in phosphate buffers at 25°. At the same pH this mobility was reduced to 2.7×10^{-5} at an ionic strength

¹ Abramson, H. A. *Electrokinetic Phenomena and their Application to Biology and Medicine*. Chemical Catalogue Co., Inc., New York. 1934.

⁶ Northrop, J. H., & Kunitz, M. *Jour. Gen. Physiol.* 7: 729. 1925.

⁷ Smith, E. E. B. *Jour. Biol. Chem.* 108: 187. 1935; 113: 473. 1936.

⁸ Scott, N. D., & Svedberg, T. *Jour. Am. Chem. Soc.* 46: 2700. 1924.

⁹ Tiselius, A. *Dissertation*, Upsala. 1930; *Trans. Faraday Soc.* 33: 524. 1937; *Biochem. Jour.* 31: 813, 1464. 1937.

¹⁰ Michaelis, L., & Airila, Y. *Biochem. Z.* 118: 144. 1921.

¹¹ Reiner, L. *Kolloid Z.* 40: 327. 1926; see also Cohn, E. J. *Physiol. Rev.* 5: 394. 1925.

¹² Cohn, E. J., Green, A. A., & Blanchard, M. H. *Jour. Am. Chem. Soc.* 59: 509. 1937. German, B., & Wyman, J., Jr. *Jour. Biol. Chem.* 117: 533. 1937.

Wyman, J., Jr. *Jour. Biol. Chem.* 127: 1. 1939.

¹³ Davis, B. D., & Cohn, E. J. *Jour. Am. Chem. Soc.* (in press).

of 0.15. At the lower ionic strength this mobility was observed at pH 6.7. A change from 0.15 to 0.02 in ionic strength thus produced a change in mobility in these systems equivalent to a change of over one pH unit. Change in ionic strength is thus not a second order phenomenon but one of first importance.

For a univalent ion the change in mobility with change in ionic strength at this temperature should on the basis of Onsager's¹⁴ development of the Debye-Hückel theory of interionic forces be:

$$u = u_0 - (\alpha u_0 + \beta) \kappa = u_0 - A' \kappa \quad (1)$$

where u_0 is the mobility at zero ionic strength, α is approximately 0.22×10^{-5} at 25° in the presence of other univalent ions, and β equals 28.9×10^{-5} . Equation (1) holds only for exceedingly dilute solutions. Using the more extended equation of Shedlovsky¹⁵ for more concentrated salt solutions

$$u = u_0 - A' \kappa + B \kappa^2 - \alpha B \kappa^3 \quad (2)$$

and putting $B = A'$, as is found to be approximately the case for many salts, yields diminution in mobilities passing from 0.02 to 0.15 in ionic strength, respectively of 5.8, 5.2, and 4.8 for K^+ , Na^+ , and Li^+ , where u_0 at this temperature is respectively 71.0, 48.5, and 37.4×10^{-5} .¹⁶ Over this ionic strength range the diminution in mobility noted for carboxyhemoglobin ($10.3 - 2.7 = 7.6$ cm²/volt-sec) was only slightly greater than that for a univalent ion in the presence of other univalent ions, and no greater than that for a bivalent ion in the presence of univalent and bivalent ions, although the valence of hemoglobin at this pH lies, as we have seen, between 12 and 16.

For the mobility of a large molecule of radius r the electrophoretic term $\beta \kappa$ of equation (1) should be divided by $(1 + \kappa r)$,¹⁷ since κr will under these circumstances no longer be small in comparison with unity. This factor is that by which u_0 is divided in the studies of Moyer and Abramson.¹⁸ Introducing it into equation (1), and neglecting the term in α , we have

$$u = u_0 - \beta \kappa / (1 + \kappa r) \quad (3)$$

¹⁴ Onsager, L. *Physik. Z.* **27**: 388. 1926; **28**: 277. 1927.

¹⁵ Shedlovsky, T. *Jour. Am. Chem. Soc.* **54**: 1405, 1411. 1932.

¹⁶ MacInnes, D. A., Shedlovsky, T., & Longworth, L. G. *Jour. Am. Chem. Soc.* **54**: 2758. 1932; *Chem. Rev.* **13**: 29. 1933.

¹⁷ MacInnes, D. A. *The Principles of Electrochemistry*. Reinhold Publishing Corp., New York. 1939.

¹⁸ Moyer, L. S. *Cold Spring Harbor Symp.* **6**: 228. 1938.

Moyer, L. S., & Abramson, H. A. *Jour. Biol. Chem.* **123**: 391. 1938.

Assuming r for carboxyhemoglobin to be 27 Å., u_0 according to this equation would be 29.8 at pH 5.65 and β would be 314, or approximately tenfold as great as for a univalent ion according to equation (1), and somewhat greater than for a univalent ion according to equation (2).

Not only the observed mobility, u , but the apparent isoelectric point is a function of the ionic strength. If our measurements be analyzed at constant pH intervals from the apparent isoelectric point, on the assumption that the influence of ionic strength on protein ions of equal valence is then considered, the values both of A' in equation (1) and β in equation (3) are much smaller. The ratio A'/u_0 is under these circumstances approximately 3.2 and that of β'/u_0 9.7 over a considerable pH range.

One must concede that in the case of proteins u_0 may have only theoretical significance because of ion association in dilute solution. None the less one must stress the influence of ionic strength, regardless of the form of equation employed, in reducing electrophoretic mobility.

A large number of measurements that have been made for this and other proteins, at 25° and at 4°, in this and other buffers, are under investigation so as to yield a more adequate foundation relating mobility to the net charge at the ionic strengths at which such measurements are generally made.

AMERICAN CITIES AND STATES: VARIATION AND CORRELATION IN INSTITUTIONS, ACTIVITIES, AND THE PERSONAL QUALITIES OF THE RESIDENTS*

By EDWARD L. THORNDIKE

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VARIATION

This paper reports certain features of a study of American cities and states, using the methods which have proved valuable in the study of individual persons. The items of fact listed below have been obtained for each city in the United States having over 30,000 population in 1930, but excluding the cities over 500,000 (except Milwaukee which was retained for special reasons) and the three resort cities Atlantic City, Miami, and St. Petersburg. In some instances where the item was not discoverable, it has been estimated on the basis of correlated items for the city in question or of the same item in cities much like the city in question. There are less than three such estimated scores per thousand. A few of the officially reported facts seem surely erroneous, and in about one case out of four thousand I have replaced an extreme measure of doubtful accuracy by an estimated measure. The distributions, correlation coefficients, and conclusions of this paper are not altered appreciably by using these procedures. Many of the items (those preceded by an S in the list below) have also been obtained for each of the forty-eight states.

List of the Facts Studied

1. Population in 1930. 1930 Statistics of Population.
2. Population in 1900. 1900 Statistics of Population.
- S 3. Recent Growth: Item 1 divided by Item 2.
- S 4. Latitude.
5. Longitude.

6. Estimated per capita value of private property computed as the assessed valuation divided by the rate customary in the city in question. Financial Statistics for Cities for 1930, Table 23.
7. The same as Item 6 except that real estate only is used.
8. Reported total paid for interest divided by reported total public indebtedness. Financial Statistics of Cities for 1930, pp. 500 f. and 430 f.
9. Density of population: persons per acre. Computed from data in Financial Statistics of Cities for 1930, Table 1.
10. Natural advantages; average of two competent estimates.
- S 11. Per capita value of all public property (excluding streets, pavements and sewers), minus public indebtedness. Computed from data in Financial Statistics of Cities for 1930, and in Financial Statistics of States for 1930.
- S 12. Per capita value of schools, libraries, museums, parks, hospitals, and asylums owned by the public. Computed from data in Financial Statistics of Cities for 1930, and Financial Statistics of States for 1930.
- S 13. Gross debt incurred for general departments and municipal service enterprises. Financial Statistics of Cities for 1930, Table 20, Section II.
14. Gross debt incurred for public service enterprises and investments. Same source as for item 13.
- S 15. Net debt. Same source as for item 13.
- S 16. The quotient of the value of public property in schools, libraries, museums, and parks divided by the value of public property used for other municipal purposes (general, government, police, fire, health, sanitation, and care of highways). Computed from data in Financial Statistics of Cities for 1930.
- S 17. Per capita acreage of parks. Bulletin 565, U. S. Bureau of Labor Statistics.
- S 21. Percentage of persons 16 or 17 years old attending school. 1930 Census, Statistics of Population, vol. 2, pp. 1112 f., 1151 f. and 1171 f.
- S 22. Percentage of persons 18 to 20 years old attending school. 1930 Census, Statistics of Population, vol. 2, pp. 1112 f., 1151 f., and 1161 f.
23. Average salary of high-school teachers. Computed from data in the Biennial Survey of Education, 1928-30, U. S. Department of the Interior, Office of Education, vol. II, pp. 142 f. and 251 f.
24. Average salary of elementary-school teachers. Same source as for Item 23.
- S 24a. Average salary of all teachers and supervisors. Items 23 and 24 could not be obtained separately for states. P. 56 f. of same source as for Item 23.
- S 25. Per capita circulation of *Better Homes and Gardens*, *Good Housekeeping* and *The National Geographic Magazine*. The circulations are for Dec. 27, March 29 and June 28, respectively as reported in "Circulation of fifteen leading magazines" by the Marketing Division of the International Magazine Co. The 1930 populations are used as divisors. The 1934 "Circulation Count" of *Better Homes and Gardens* (Meredith Publishing Co.), the 1934 "Good Housekeeping Circulation" and the 1935 "Net Paid Distribution of the National Geographic Magazine" were also used in the case of the cities.
- S 26. Per capita circulation of the *Literary Digest*, average of two years (1926 as reported by the Marketing Division of the International Magazine Co., divided by the geometric mean of the 1920 and 1930 populations; 1930 as reported in "The Lord of the Manor.") For states only the latter was used.
27. Per capita circulation of fifteen important magazines (not available for all cities). Same source as for item 25.

28. Per capita circulation (approximate) of *Modern Screen*, *Radio Stars*, and *Modern Romances*. Data from "Modern Magazines" for 1935 divided by 1930 population. This is a booklet with no author or publisher stated. It is probably obtainable from the advertising department of any one of the three magazines.
29. Approximate per capita circulation of all MacFadden magazines. 1936 circulation of *Liberty*, *True Story*, MacFadden Women's Group. Division of Marketing and Research, MacFadden Publications.
- S 30. Per capita number of pupils graduating from public high-schools in 1935. The gross numbers graduating were supplied by the courtesy of the Office of Education in the Department of the Interior. The 1930 populations were used as divisors.
- S 31. Per capita number of illiterates (all).
32. Per capita number of illiterates aged 10-14.
- S 33. Per capita number of illiterates aged 15-24.
34. Per capita number of illiterates aged 25-34.
35. Per capita number of illiterates aged 35-44.
36. Per capita number of illiterates aged 45-54.
37. Per capita number of illiterates aged 55-64.
38. Per capita number of illiterates aged 65 and over.
Items 31-38 are taken from the 1930 Census, Statistics of Population, Vol. 2, pp. 1238 f., 1275 f. and 1303 f.
39. Per capita number of volumes owned by the library.
40. Per capita number of borrowers from the library.
41. Per capita library circulation.
The data for items 39, 40 and 41 in certain cities are available in the Bulletin of the American Library Association, Vol. 31, No. 4, April 1937. Those for the other cities were supplied by the courtesy of the American Library Association.
- 43, 44. Per capita expenditures for highways and light.
45. Per capita expenditures for sanitation.
46. Per capita expenditures for general government.
47. Per capita expenditures for police.
48. Per capita expenditures for fire.
49. Per capita expenditures for health.
50. Per capita expenditures for public safety.
51. Sum of items 43 to 51.
Items 43 to 51 are from Financial Statistics of Cities, 1930.
53. Per capita expenditures for schools.
Financial Statistics of Cities for 1930.
- S 53a. Per capita expenditures for schools (including building costs).
Statistical Abstract of the United States, 1935, Table 117.
- S 54. Per capita expenditure for teachers' salaries. Computed from data in the Biennial Survey of Education for 1928-1930, Bulletin 20, Office of Education, U. S. Department of Interior.
- S 55. Per capita expenditures for textbooks and class-room supplies. Same source as for Item 54.
- S 56. Per capita expenditures for libraries and museums. Computed from data in Financial Statistics of Cities for 1930, and Financial Statistics of States for 1930.

- S 57. Per capita expenditures for recreation. Same sources as for Item 56.
- S 59. Approximate per capita wholesale sales for 1933. Consumer Market Data Handbook, Market Research Series No. 15, U. S. Department of Commerce, 1936.
- S 60. Approximate per capita sales of all retail stores for 1933. Same source as for item 59.
- S 61. Per capita sales of all retail stores for 1930.
- S 62. Per capita sales of all retail food stores.
63. Per capita sales of all retail stores except food stores.
- S 64. Per capita sales of all retail cigar stores.
- S 65. Per capita sales of all retail drug stores.
67. Per capita sales of all retail auto stores.
- S 68. Per capita sales of all retail radio and music stores.
- S 69. Per capita sales of all retail book stores.
70. Per capita sales of all retail florists.
71. Per capita sales of all jewelry stores.
72. Per capita sales of all music (without radio) stores.
73. Per capita sales of all newsdealers.
- S 74. Per capita sales of all second-hand stores.
Items 61-74 are computed from data in the 1930 Census of Distribution, Vol. 1, parts II and III.
- S 75. Approximate per capita service, amusements, and hotel receipts for 1933. Consumer Market Data Handbook. Market Research Series No. 15. U. S. Department of Commerce, 1936.
76. Approximate per capita postal receipts for 1933. Same source as for Item 75.
- S 77. Percentage which sales of food stores are of total retail sales.
78. Percentage which sales of auto stores are of total retail sales.
- S 79. Percentage which sales of radio and music stores are of total retail sales.
80. Percentage which sales of book stores are of total retail sales.
- S 81. Percentage which sales of cigar stores are of total retail sales.
- S 82. Percentage which sales of drug stores are of total retail sales.
83. Percentage which sales of florists are of total retail sales.
84. Percentage which sales of jewelry stores are of total retail sales.
85. Percentage which sales of music (no radio) stores are of total retail stores.
86. Percentage which sales of newsdealers are of total retail sales.
87. Percentage which sales of second-hand stores are of total retail sales.
- S 88. Percentage which sales of "single" stores are of total retail sales.
- S 89. Percentage which sales of "chain" stores (local, sectional, or national) are of total retail sales.
90. Per capita number of book stores.
91. Per capita number of cigar stores.
92. Per capita number of drug stores.
93. Per capita number of jewelry stores.
Items 77 to 93 are quoted or computed from the 1930 Census of Distribution, Vol. 1, parts II and III.
- S 97. Approximate per capita number of homes wired for electricity in 1933. Consumer Market Data Handbook. Market Research Series No. 15, U. S. Department of Commerce, 1936.
98. Per capita domestic installations of electricity. The number reported in

Markets and Quotas, Curtis Publishing Co., 1932, divided by the 1930 population. The original authorities are the National Electric Light Association and the U. S. Department of Commerce.

- S 99. Per capita domestic installations of gas. Same as for Item 98. The original authority is the American Gas Association.
- S 100. Per capita number of Ford automobiles. Computed from data in Markets and Quotas, Curtis Publishing Company, 1932.
- S 101. Per capita number of automobiles other than Fords. Same source as Item 100.
- S 102. Per capita number of automobiles: the sum of items 100 and 101.
- S 103. Per capita number of telephone subscribers. The Lord of the Manor, published by the Literary Digest.
- S 104. Per capita number of radio sets. 1930 Census, Population, Vol. VI, Tables 59, 76, and 79.
- S 106. Per capita number of homes owned. 1930 Census, Population, Vol. VI, Table 41 and Table 4 for each state.
- 106a. Percentage of homes owned by their occupants.
- 107. Rarity of extreme poverty, measured by the rent paid (or equivalent if the home is owned) by the 5 percentile family (i. e., the rental less than which is paid by 5%, and more than which is paid by 95% of the community's families).
- 107a. Rarity of extreme poverty, measured by the percentage of families living in homes renting for less than \$10 a month.
- 108. Rarity of less extreme poverty, measured by the rent paid (or equivalent) by the 10 percentile family.
- 109. Median family rental (or equivalent if the home is owned).
- 110. The rent paid by the 75 percentile family.
- 111. The rent paid by the 90 percentile family.
- 112. The rent paid by the 95 percentile family.
- 113. Approximate average family rental.
Items 107 to 113 are computed from data in the 1930 Census, Statistics of Population, Vol. VI, Table 67 and Table 7 for each state. In the states similar measures (Tables 45 and 46) were obtained for the non-farm homes only.
- 114 to 118. Variability in income as indicated by variability in amounts paid for rental (or equivalent if the home is owned).
- 114. Item 112 minus item 107.
- 115. Item 112 divided by item 107.
- 116. Item 111 minus item 108.
- 117. Item 111 divided by item 108.
- 118. Per capita number of homes over \$100 a month plus per capita number of homes under \$10 a month. Same source as Items 107 to 113.
- 119. Variability in income as indicated by the sum of the approximate percentage of families reporting income tax and the approximate percentage of families *not* supplied with electricity. Computed from items 233, 97, and 98.
- 120. 90 percentile rental minus 50 percentile rental.
- 120a. 95 percentile rental minus 50 percentile rental.
- 120b. 50 percentile rental minus 5 percentile rental.
- 120c. 50 percentile rental minus 10 percentile rental.

- 122. Approximate per capita value of church property.
- 123. Approximate per capita annual expenditures of religious bodies.
- S 124. Approximate percentage of the population who are church members aged 13 or over.
- S 125. Approximate percentage of the population who are members, aged 13 or over, of Roman Catholic churches.
- S 126. Approximate percentage of the population who are members, aged 13 or over, of Jewish congregations.
- S 127. Approximate percentage of the population who are members, aged 13 or over, of Unitarian, Universalist, or Christian Science Churches.
Items 122 to 127 are computed from the data in the 1926 Census of Religious Bodies, using the estimated 1925 population as a divisor.
- S 128. Males per 1000 females.
- S 129. Number of births per 1000 population.
- S 130. Number of births per 1000 females age 20 to 44. Computed from item 129 and item 150.
- S 131. Infant death-rate, average of 1926 to 1934, reversed so that low rates are scored + and high rates —. Statistical Report of Infant Mortality for 1934. American Child Health Association.
- S 132. General death-rate (average of 1930, 1933, 1934) reversed as in Item 2.
- S 133. Per capita deaths from syphilis, average of four years.
- S 134. Per capita deaths from typhoid, average of six years.
- S 135. Per capita deaths from diabetes, average of six years.
- S 136. Per capita deaths from appendicitis, average of six years.
- S 137. Per capita deaths from puerperal diseases, average of six years.
Items 132 to 137 were computed from data in Mortality Statistics, Bureau of the Census, Vols. for 1928 to 1933.
- 138. Per capita deaths from gonococcus infections, etc., average of three years. Computed from data furnished by the Division of Vital Statistics of the U. S. Census Bureau.
- S 140. Median size of family in 1930. 1930 Census of Population, Vol. VI, Table 66 and Table 21 for each state.
- S 141. Average size of family in 1900, 1910 and 1920. 1920 Census of Population, Vol. 2, Table 4.
- S 142. Change in size of family. Average size in 1920 minus average size in 1900.
- S 143. Percentage which native white families are of all families.
- S 144. Percentage which foreign born white families are of all families.
- S 145. Percentage which negro families are of all families.
Items 143 to 145 are from the 1930 Census of Population, Vol. VI, Table 63 and Table 21 for each state.
- 146. Percentage of the population under 15.
- S 147. Percentage of the population under 10.
- S 147a. The same for males.
- S 147b. The same for females.
- S 148. Percentage of the population 65 and over.
- S 149. Percentage of males 20 to 39.
- S 149a. Percentage of males 40 to 64.
- S 150. Percentage of females 20 to 44.
- S 150a. Percentage of females 40 to 64.

- S 150b. Percentage of the population 10 to 14.
- S 150c. Percentage of the population 15 to 19.
Items 146 to 150c are computed from the 1930 Census, Population, Vol. II, Tables 33, 38 and 39.
151. Percentage of males 10 yr. and over gainfully employed. 1930 Census, Statistics of Population, Vol. IV, Tables 24 and 57.
152. Percentage of females 10 yr. and over gainfully employed. Same source as for item 151.
- S 153. Percentage of males 10 to 15 years old *not* gainfully employed.
- S 154. Percentage of females 10 to 15 years *not* gainfully employed.
- S 155. Per capita number of married women gainfully employed. 1930 Census of Population, Vol. IV, Table 10 and Table 5 for each state.
- S 156. Per capita number of wage earners in factories. Computed from data in Markets and Quotas, Curtis Publishing Company, 1932.
157. Unemployment in 1930. Persons per capita out of a job and looking for a job. U. S. Census, 1930; Unemployment, Vol. 1, Table 14, pp. 24-41, and Table 10 for individual states.
158. Unemployment in 1930. Persons per capita having jobs but laid off though able and willing to work. Same source as for item 157.
- S 162. Per capita number of male clergy.
- S 163. Per capita number of female clergy.
- S 164. Per capita number of male physicians.
- S 165. Per capita number of female physicians.
- S 166. Per capita number of male nurses.
- S 167. Per capita number of female nurses.
- S 168. Per capita number of male domestic servants.
- S 169. Per capita number of female domestic servants.
- S 171. Per capita number of male teachers.
- S 172. Per capita number of female teachers.
- S 173. Per capita number of male musicians.
- S 174. Per capita number of female musicians.
- S 177. Per capita number of male dentists.
- S 178. Per capita number of female dentists.
- S 179. Per capita number of male lawyers.
- S 180. Per capita number of female lawyers.
- S 181. Per capita number of male actors.
- S 182. Per capita number of female actors.
- S 183. Per capita number of male sculptors, artists and teachers of art.
- S 184. Per capita number of female sculptors, artists and teachers of art.
- S 185. Per capita number of male public employees.
- S 186. Per capita number of female public employees.
- S 187. Per capita number of male professional workers (exclusive of teachers).
- S 188. Per capita number of female professional workers (exclusive of teachers).
- S 189. Per capita number of male police.
- S 190. Per capita number of male architects.
- S 191. Per capita number of male designers.
- S 192. Per capita number of male osteopaths.
- S 193. Per capita number of male technical engineers.
- S 194. Per capita number of male veterinary doctors.

- 195. Per capita number of female police.
- 196. Per capita number of female architects.
- S 197. Per capita number of female designers.
- S 198. Per capita number of female osteopaths.
- 199. Per capita number of female technical engineers.
- 200. Per capita number of female veterinary doctors.
- S 201. A weighted composite of the per capita numbers of physicians, nurses and teachers minus a score for the per capita number of male domestic servants. The first three have approximately equal weight, and the fourth approximately as much weight as the first three combined.
- S 202. Number of female clergy divided by the number of male clergy.
- S 203. Sum of items 195, 196, 197 and 200.
- S 204. Ratio of female to male dentists.
- S 205. Ratio of female to male lawyers.
- S 206. Ratio of female to male teachers.
- S 207. Ratio of female to male actors.
- S 207a. Ratio of female to male sculptors, artists and teachers of art.
- S 207b. Ratio of female to male public employees.
- S 207c. Ratio of female to male professional workers (exclusive of teachers).
- S 207d. Ratio of male dentists to male lawyers.
- Items 162 to 207d are computed from data given in the 1930 Census, Statistics of Population, Vol. IV, Table 5 for each state, and Table 4 for some states.
- S 208. Percentage of the population listed in Who's Who for 1930. Computed from data in the geographical index in Who's Who, 1930.
- S 209. Percentage of the population listed in Who's Who for 1910. Computed from data in the geographical index in Who's Who, 1910.
- S 210. Increase in the percentage in Who's Who, 1910 to 1930 (Item 208 divided by Item 209).
- S 211. Percentage of the population enrolled as members of the American Association for the Advancement of Science. Data from Proceedings of the A. A. A. S., vols. 82 to 87.
- 220. Average salary of full-time workers in all retail stores. 1930 Census, Distribution, Vol. I, parts II and IV.
- 221. Average salary of full-time workers in national chain stores. Same source as item 220.
- 222. Average of items 220 and 221.
- S 223. Average wage in manufacturing plants. (The two years 1929 and 1931 were used.) Biennial census of manufactures.
- 224. Salary of a policeman (after 5 years of service).
- 225. Salary of a fireman (after 5 years of service). The data for items 224 and 225 were furnished for 258 cities by the courtesy of their mayors.
- 229 to 232. Per capita income of citizens, by various commercial estimates of the total income of the city.
- 229. By the estimate in Markets and Quotas, The Curtis Publishing Co., 1932.
- 230. By the estimate of "Total Income," in National Markets and National Advertising, Advertising Department of the Crowell Publishing Co., 1927.
- 231. By the "Marginal Income" of the same publication.
- 232. By the "Per Capita Effective Income" of the same publication.

- S 233. Approximate per capita number of income tax returns: average number for 1930 and 1931, divided by the 1930 population. Statistics of Income for 1930, U. S. Treasury Department, 1932, and the same for 1931.
234. Approximate per capita number of income tax returns for 1933. U. S. Treasury Publication, No. 2108.
- S 235. Approximate per capita number of income-tax returns of incomes exceeding \$5000. Estimated from the data for counties reported in Markets and Quotas, Curtis Publishing Co., 1926, in the case of the cities.
237. Per capita value added to product by manufacture.
238. Per capita bank deposits. Markets and Quotas. The Curtis Publishing Co., 1932.
- S 241. Per capita deaths from homicides: Average of six years.
- S 242. Per capita deaths from suicide: Average of six years.
- S 243. Per capita deaths from automobil accidents: Average of six years. Items 241 to 243 are computed from data in Mortality Statistics, vols. for 1928-1933, Bureau of the Census.
244. Per capita illegitimate births: Average of three years. Computed from data furnished by the Division of Vital Statistics of the U. S. Census Bureau.
- S 248. A weighted composite of per capita membership, current expenses and value of property of the Y. M. C. A. Computed from data in the Y. M. C. A. Year Book and Official Roster for 1930.
- S 249. Per capita membership of the Boy Scouts. Twenty-first Annual Report of the Boy Scouts of America, 1930. House document No. 794 of the 71st Congress, 3rd session.
250. Church expenditures for outside benevolences divided by their expenditures for their own minister, music, maintenance of plant, etc., using facts for the Baptist, Congregationalist, Christian, Methodist, and Presbyterian denominations, but excluding churches with Negro or chiefly foreign memberships. Computed from data in the Year Book of the Congregational Church, the Presbyterian Church in the U. S. A., General Assembly, 1930, Part I, various Annuals of the Baptist Church, usually for separate states, the Minutes of various Annual Conferences of the Methodist Episcopal Church, Yearbook of the Christian Church, and documents made available to us in the offices of the Methodist Episcopal Church South, and of the Southern Baptist Church.
- S 251. Per capita membership in Kiwanis Clubs. Computed from data furnished by the department of records of Kiwanis International.
- S 252. Per capita membership in Rotary Clubs. Computed from data in the Official Directory of Rotary International, 1929, 1930 and 1931.
- 255 to 262. Percentages of the total expenditures for maintenance and operation devoted to various purposes.
255. Percentage of total expenditures devoted to general government.
256. Percentage of total expenditures devoted to police.
257. Percentage of total expenditures devoted to health.
258. Percentage of total expenditures devoted to sanitation.
259. Percentage of total expenditures devoted to charities, hospitals and corrections.
260. Percentage of total expenditures devoted to schools.
- S 261. Percentage of total expenditures devoted to libraries.

262. Percentage of total expenditures devoted to recreation.
Items 255 to 262 are taken from Financial Statistics of Cities for 1930, Table 14, and Financial Statistics of States, Table 13.
263. Employment of a city manager.
264. Provision for civil service examinations.
265. Provision for city planning.
266. Provision for obtaining and recording vital statistics.
267. Provision for public convenience stations.
268. Provision for a probation system.
269. Special financial aid to mothers.
The facts for items 263-269 were as found in Table 12 of Financial Statistics of Cities.
270. A composite score summing credits given for items 263 to 270 with consideration of the size of the city.
271. Sum of credits for per capita amounts spent on civil service, city planning, vital statistics and public convenience stations.
- 274 to 296. Median prices as per the advertisements in the local newspapers during the week September 13 to September 18, 1937 for home rentals, house sales and various commodities and services.

Variations among Cities

The variations among cities in most of these items is very great, as will appear from the samples presented in TABLE 1. An examination of these and of over two hundred other distributions not printed here shows that with hardly an exception they are continuous and uni-modal. They are much less often symmetrical than are distributions of biological and psychological characteristics in individuals. The mode is sometimes at a superior condition in the trait in question with a long tail down to very inferior conditions. Such is the case, for example, with infant mortality, deaths from typhoid, homicides, and child labor. The mode is sometimes at an inferior condition with a long tail up to extremely good conditions, for example in the per capita amount spent for teachers' salaries, or for libraries, the per capita value of public buildings, the support of Y.M.C.A., the average rent per family, the per capita membership in the American Association for the Advancement of Science, and the per capita circulation of public-library books.

Fifty-five of the cities adjoin larger cities, usually as residential or industrial suburbs. Forty of them are situated in the states of the Old South (Virginia, North Carolina, South Carolina, Georgia, Florida, Kentucky, Tennessee, Alabama, Mississippi, and Louisiana), and in certain respects deserve study by themselves. The two hundred that remain form a more homogeneous group of urban communities, and I shall in general compute correlations for both the 295 and the 200 cities.

TABLE I
THE NATURE AND AMOUNT OF VARIATION IN 295 CITIES
Q = quantity F = frequency

ITEM 54		ITEM 56		ITEM 12		ITEM 106a		ITEM 23		ITEM 21	
Per capita expenditures for teachers' salaries		Per capita expenditures for libraries and museums		Per capita value of schools, libraries, museums, parks, hospitals & asylums owned by the public		Percentage of homes owned by the occupants		Average high-school teacher's salary		Percentage of persons 16-17 years old attending school	
Q	F	Q	F	Q	F	Q	F	Q	F	Q	F
\$3.00-\$3.99	1	\$.00-.09	10	\$20.00-\$29.99	1	14-15.9	1	\$1050-\$1149	3	26-28.9	1
4.00-4.99	3	.10-.19	27	30.00-39.99	7	16-17.9	1	1150-1249	6	29-31.9	9
5.00-5.99	8	.20-.29	32	40.00-49.99	17	18-19.9	4	1250-1349	6	32-34.9	1
6.00-6.99	9	.30-.39	31	50.00-59.99	25	20-21.9	4	1350-1449	9	35-37.9	4
7.00-7.99	19	.40-.49	43	60.00-69.99	50	22-23.9	4	1450-1549	13	38-40.9	10
8.00-8.99	28	.50-.59	34	70.00-79.99	39	24-25.9	5	1550-1649	11	41-43.9	10
9.00-9.99	45	.60-.69	28	80.00-89.99	37	26-27.9	5	1650-1749	14	44-46.9	16
10.00-10.99	41	.70-.79	24	90.00-99.99	36	28-29.9	4	1750-1849	18	47-49.9	24
11.00-11.99	33	.80-.89	24	100.00-109.99	20	30-31.9	7	1850-1949	27	50-52.9	19
12.00-12.99	26	.90-.99	17	110.00-119.99	16	32-33.9	14	1950-2049	36	53-55.9	21
13.00-13.99	22	1.00-1.09	10	120.00-129.99	16	34-35.9	18	2050-2149	26	56-58.9	31
14.00-14.99	24	1.10-1.19	5	130.00-139.99	6	36-37.9	23	2150-2249	27	59-61.9	32
15.00-15.99	8	1.20-1.29	2	140.00-149.99	7	38-39.9	17	2250-2349	21	62-64.9	11
16.00-16.99	7	1.30-1.39	3	150.00-159.99	4	40-41.9	13	2350-2449	12	65-67.9	24
17.00-17.99	6	1.40-1.49	2	160.00-169.99	5	42-43.9	22	2450-2549	20	68-70.9	21
18.00-18.99	4	1.50-1.59	1	170.00-179.99	2	44-45.9	19	2550-2649	8	71-73.9	14
19.00-19.99	4	1.60-1.69	1	180.00-189.99	2	46-47.9	18	2650-2749	13	74-76.9	14
20.00-20.99	1	1.70-1.79	1	190.00-199.99	1	48-49.9	16	2750-2849	6	77-79.9	7
21.00-21.99	1	1.80-1.89	1	200.00-209.99	1	50-51.9	25	2850-2949	5	80-82.9	7
22.00-22.99				210.00-219.99	2	52-53.9	22	2950-3049	2	83-85.9	7
23.00-23.99				220.00-229.99	1	54-55.9	18	3050-3149	1	86-88.9	12
24.00-24.99				230.00-239.99		56-57.9	14	3150-3249	3	89-91.9	6
25.00-25.99				240.00-249.99		58-59.9	13	3250-3349	4	92-94.9	4
26.00-26.99				250.00-259.99	1	60-61.9	4	3350-3449	1		
27.00-27.99	1					62-63.9	5	3450-3549	1		
						64-65.9	2	3550-3649	2		
						66-67.9	1	3650-3749			
						68-69.9	1	3750-3849			
								3850-3949	1		

TABLE 1—(continued)

Variations among States

The forty-eight states are not as instructive or "natural" units as the cities, but each of them does have a certain individuality in population, economic life and customs, as well as in government and laws. In any case the facts for them are valuable as independent evidence concerning certain general problems.

The variation among the states in important features of life and welfare is very great. Its amount can be realized easily by glancing down column after column of TABLE 2 which presents some of the facts about the states found in various sources or computed from them. States are large enough to guarantee that if the records are impartial the comparison of even the two extremes among the forty-eight will be dependable. The full table of which TABLE 2 gives sample columns shows that the population gain from 1900 to 1930 of California was over a thousand times as great as Vermont's, that the public property balance per capita of Arkansas was reported to be about as far in the red as that of the best off state was on the plus side, that the reported per capita value of public property in schools, parks, etc. was over ten times as great in Delaware as in Georgia, that the percentage of persons aged 16 or 17 in school was nearly twice as high in California and Utah as in Georgia, Maryland and Rhode Island, that the average salary of teachers and supervisors in public schools was nearly four times as high in New York as in Mississippi, and so on through the list.

TABLE 2
VARIATION AMONG STATES IN EIGHTY-TWO IMPORTANT FEATURES OF
LIFE AND WELFARE

	Percentage of persons 16 or 17 years old attending school.	Percentage of persons 18-20 years old attending school.	Average salary of teachers and supervisors.	Circulation of three reputable magazines, per 10,000.	Circulation of Literary Digest, per 10,000.	Graduates from senior high-school in 1934, per 100,000.	Percentage of illiterates for total population.	Percentage of illiterates for ages 15-24.	City and county parks: acres, per 100,000.
	21	22	23 & 24	25	26	30	31	33	17
1. Ala.	51.5	18.0	\$ 792	91	63	281	12.6	7.7	66
2. Ariz.	56.6	22.6	1637	281	144	610	10.1	7.2	3371
3. Ark.	59.5	22.6	673	115	53	402	6.8	3.8	39
4. Calif.	82.1	32.7	2123	470	208	842	2.6	1.1	652
5. Colo.	63.2	28.4	1453	309	147	822	2.8	1.3	1654
6. Conn.	47.3	17.8	1812	474	144	729	4.5	0.6	6223
7. Del.	52.0	18.1	1570	346	129	503	4.0	1.1	337
8. Fla.	55.0	19.7	876	335	118	362	7.1	5.2	355
9. Ga.	43.6	14.3	684	133	59	220	9.4	6.3	115
10. Idaho	72.3	32.9	1200	431	161	1229	1.1	0.3	72
11. Ill.	57.1	19.9	1630	359	105	689	2.4	0.5	703
12. Ind.	62.4	21.7	1466	323	92	877	1.7	0.5	245
13. Iowa	63.9	25.1	1094	407	98	1008	0.8	0.3	227
14. Kan.	66.4	28.9	1159	323	102	996	1.2	0.3	99
15. Ky.	40.2	19.1	896	143	59	401	6.6	3.7	124
16. La.	40.5	17.8	941	103	69	465	13.5	8.5	106
17. Me.	59.8	23.1	942	358	140	706	2.7	1.2	66
18. Md.	42.2	16.2	1518	277	94	489	3.8	1.5	225
19. Mass.	59.5	25.0	1875	397	143	753	3.5	0.5	344
20. Mich.	61.7	20.2	1534	347	116	691	2.0	0.4	287
21. Minn.	57.0	24.4	1251	356	126	826	1.3	0.3	441
22. Miss.	59.6	23.1	620	92	50	341	13.1	8.6	60
23. Mo.	51.6	20.0	1235	252	80	576	2.3	0.9	255
24. Mont.	67.9	29.6	1215	439	189	995	1.7	0.5	172
25. Neb.	63.2	24.7	1077	366	106	1011	1.2	0.3	528
26. Nev.	75.7	31.2	1483	389	213	882	4.4	1.6	370
27. N. H.	55.5	22.2	1254	383	161	713	2.7	0.6	242
28. N. J.	47.6	16.0	2113	362	141	621	3.8	0.7	329
29. N. M.	57.4	22.1	1113	207	91	545	13.3	8.3	2
30. N. Y.	59.4	20.4	2493	339	131	561	3.7	0.3	367
31. N. C.	49.3	19.2	873	146	60	646	10.0	6.6	68
32. N. D.	58.4	25.1	900	267	97	949	1.5	0.5	64
33. Ohio	67.7	22.8	1665	382	111	780	2.3	0.5	399
34. Okla.	63.2	25.8	1072	203	71	716	2.8	1.4	482
35. Ore.	72.8	33.3	1612	595	180	953	1.0	0.4	590
36. Pa.	48.6	17.2	1620	326	110	639	3.1	0.5	196
37. R. I.	40.4	17.0	1437	305	112	441	4.9	0.7	188
38. S. C.	46.7	18.1	788	108	48	305	14.9	11.7	85
39. S. D.	62.8	27.9	956	291	107	924	1.2	0.3	113
40. Tenn.	53.2	19.9	902	136	61	471	7.2	4.3	208
41. Tex.	57.2	19.8	924	168	101	624	6.8	5.1	352
42. Utah	80.9	32.7	1330	325	173	1176	1.2	0.7	881
43. Vt.	55.7	21.3	963	493	161	605	2.2	0.6	201
44. Va.	48.3	18.1	861	206	93	501	8.7	5.4	130
45. Wash.	76.8	33.6	1556	575	189	1041	1.0	0.3	581
46. W. V.	49.8	20.3	1023	199	72	624	4.8	2.2	71
47. Wis.	63.4	21.5	1399	304	101	841	1.9	0.4	322
48. Wyo.	68.4	26.5	1239	493	154	1073	1.6	0.9	383

TABLE 2—(continued)

	Retail sales, music and radio stores, per capita.	Retail sales, second-hand stores, per capita.	Approximate service, amusements, and hotel receipts.	Retail food-sales as a percentage of all retail sales.	Radio and music store sales as a percentage of all retail sales.	Cigar store sales as a percentage of all retail sales.	Drug store sales as a percentage of all retail sales.	"Single" store sales as a percentage of all retail sales.	Chain store sales as a percentage of all retail sales.
	68	74	75	77	79	81	82	88	89
1. Ala.	\$.73	\$.40	\$ 6.60	16.26	.37	.24	4.44	59.49	13.94
2. Ariz.	.63	2.16	18.30	19.41	.14	.76	3.66	62.76	21.23
3. Ark.	.96	.49	6.50	13.17	.43	.20	4.19	64.54	11.10
4. Calif.	4.33	2.81	40.00	20.46	.77	1.06	3.61	60.75	22.54
5. Colo.	2.84	2.63	21.80	20.09	.63	.72	3.92	67.60	15.40
6. Conn.	2.20	.77	23.10	26.56	.46	.74	2.97	68.23	21.06
7. Del.	3.44	1.84	19.80	25.32	.64	2.00	2.43	70.60	15.72
8. Fla.	1.17	1.15	18.10	22.37	.34	.50	4.88	69.21	17.12
9. Ga.	.76	1.02	9.30	18.94	.35	.30	4.39	64.74	16.29
10. Idaho	1.87	.88	15.70	17.08	.49	1.55	3.28	62.76	20.54
11. Ill.	2.61	1.59	33.10	22.81	.54	.98	3.55	59.57	22.42
12. Ind.	1.56	1.08	14.70	22.20	.41	.87	3.81	68.07	18.62
13. Iowa	1.16	.74	16.60	16.04	.29	.94	3.40	62.89	14.56
14. Kan.	1.60	1.10	13.70	17.32	.41	.43	4.12	62.90	15.52
15. Ky.	.58	.50	10.90	20.57	.26	.28	3.88	63.21	14.81
16. La.	1.00	1.06	12.20	16.93	.44	.42	4.42	70.57	12.68
17. Me.	1.15	.49	17.00	23.65	.30	.48	3.33	72.60	13.16
18. Md.	1.44	1.74	22.80	27.09	.38	.52	3.08	67.10	18.11
19. Mass.	1.25	1.11	27.90	27.05	.26	.69	3.13	60.55	22.82
20. Mich.	2.79	1.59	19.40	22.09	.61	.71	3.54	64.18	22.37
21. Minn.	1.58	.80	20.80	18.53	.39	.92	2.67	63.66	13.06
22. Miss.	.34	.06	4.60	13.93	.17	.14	3.95	62.50	7.36
23. Mo.	1.79	1.54	23.70	19.01	.45	.45	4.13	59.17	17.18
24. Mont.	2.25	1.13	18.20	18.13	.50	1.86	2.88	70.18	14.81
25. Neb.	2.10	.56	18.10	15.54	.51	.75	3.62	65.69	12.52
26. Nev.	5.74	2.88	34.60	18.74	1.04	1.62	3.33	77.84	10.32
27. N. H.	1.92	.49	19.90	27.47	.48	.28	3.70	70.44	15.66
28. N. J.	2.25	1.20	25.60	30.69	.50	.88	2.74	68.03	21.42
29. N. M.	.90	.41	11.40	15.67	.32	.35	3.59	67.33	13.43
30. N. Y.	2.61	1.38	49.50	25.06	.47	1.32	2.88	63.84	22.65
31. N. C.	.65	.34	8.50	17.87	.32	.27	3.82	67.65	15.68
32. N. D.	.75	.23	11.30	13.22	.22	1.25	3.63	63.11	12.76
33. Ohio	1.78	1.60	21.50	24.00	.41	.80	3.17	65.95	21.22
34. Okla.	1.54	1.58	11.90	16.69	.47	.34	4.69	62.32	17.69
35. Ore.	1.68	2.51	23.60	21.06	.35	1.25	3.25	69.06	15.91
36. Pa.	1.84	.04	17.80	24.78	.46	1.06	2.98	63.79	20.21
37. R. I.	2.04	1.56	22.10	26.41	.44	.67	3.58	64.99	23.19
38. S. C.	.58	.43	8.20	19.43	.33	.22	3.96	68.40	12.72
39. S. D.	.67	.24	11.20	12.93	.18	.84	3.16	62.54	13.79
40. Tenn.	.45	.84	10.60	17.73	.18	.44	3.61	64.80	14.14
41. Tex.	1.39	1.21	15.50	17.06	.39	.24	4.75	63.08	15.83
42. Utah	2.53	1.48	16.00	17.16	.63	.98	3.11	66.59	16.90
43. Vt.	1.40	.81	17.40	20.90	.33	.43	2.77	73.38	10.42
44. Va.	1.00	.84	13.70	19.50	.40	.52	3.77	65.53	16.05
45. Wash.	2.66	2.43	26.60	21.47	.55	1.56	3.30	68.48	17.75
46. W. V.	1.07	.46	10.20	17.88	.41	.42	2.98	64.25	17.09
47. Wis.	1.79	.66	17.70	21.33	.42	.59	2.91	67.43	16.03
48. Wyo.	2.85	.73	22.90	17.56	.62	1.20	3.65	73.61	12.22

TABLE 2—(continued)

	Wired homes, per 1000.	Domestic installations of gas per 1000.	Ford automobiles per 10,000.	Automobiles other than Fords, per 10,000	All automobiles, per 1000.	Telephones per 10,000.	Radios per 10,000.	Homes owned per 10,000.	Estimated 5 percentile monthly rental, non-farm homes.
	97	99	100	101	102	103	104	106	107
1. Ala.	57	24	330	441	77	257	213	750	\$ 4.80
2. Ariz.	125	61	615	1372	199	436	440	1052	5.40
3. Ark.	52	37	281	348	63	328	216	910	5.10
4. Calif.	266	241	799	2240	304	1245	1473	1278	11.20
5. Colo.	164	95	803	1611	241	1120	974	1270	6.30
6. Conn.	234	193	319	1447	177	1163	1323	1065	12.60
7. Del.	160	130	588	1140	173	797	1137	1266	7.50
8. Fla.	125	62	522	915	168	482	396	1048	5.30
9. Ga.	56	31	364	476	84	341	222	669	4.90
10. Idaho	149	10	636	1297	193	766	736	1339	5.80
11. Ill.	210	188	494	1238	173	1417	1406	1157	10.00
12. Ind.	182	112	754	1400	215	1111	1085	1465	6.90
13. Iowa	161	77	1037	1561	260	1453	1249	1380	7.70
14. Kan.	152	101	969	1408	238	1250	1008	1421	6.20
15. Ky.	92	62	391	598	99	546	425	1170	5.30
16. La.	79	63	377	522	90	405	258	789	5.20
17. Me.	193	52	501	1278	178	1228	973	1504	6.40
18. Md.	169	145	445	1088	153	722	1012	1278	7.50
19. Mass.	407	216	402	1222	162	1293	1383	1032	13.00
20. Mich.	193	146	714	1434	213	945	1237	1419	9.50
21. Minn.	146	97	849	1405	225	1126	1119	1365	9.30
22. Miss.	36	10	282	326	61	230	126	747	4.50
23. Mo.	152	108	627	991	162	995	970	1269	6.80
24. Mont.	124	39	495	1295	179	653	808	1328	5.90
25. Neb.	144	70	990	1480	247	1416	1192	1317	8.20
26. Nev.	146	32	481	1577	205	884	856	1269	5.50
27. N. H.	210	88	528	1390	192	1272	1139	1393	7.10
28. N. J.	226	223	351	1389	174	965	1543	1162	16.00
29. N. M.	70	27	437	816	125	288	268	1286	4.30
30. N. Y.	237	230	293	1131	142	1192	1442	912	15.30
31. N. C.	67	14	438	555	99	294	227	882	5.10
32. N. D.	71	22	912	1110	202	731	870	1195	6.60
33. Ohio	197	187	667	1577	224	1098	1214	1368	9.80
34. Okla.	93	99	663	981	164	652	507	939	5.70
35. Ore.	204	101	548	1184	173	1198	1215	1617	6.80
36. Pa.	180	157	371	1156	153	895	1118	1246	8.90
37. R. I.	237	194	346	1264	161	1058	1375	982	11.20
38. S. C.	49	14	467	468	93	216	160	640	3.70
39. S. D.	91	31	907	1363	227	936	1028	1190	7.10
40. Tenn.	78	29	427	642	107	485	328	1032	5.30
41. Tex.	94	92	751	970	172	582	442	959	5.50
42. Utah	186	43	482	1127	161	799	938	1370	6.50
43. Vt.	182	59	439	1339	178	1265	1106	1469	6.90
44. Va.	90	44	486	706	119	445	398	1124	5.40
45. Wash.	218	57	636	1436	207	1206	1149	1569	7.30
46. W. V.	86	102	355	868	122	406	503	974	5.30
47. Wis.	178	115	617	1377	199	1175	1235	1497	10.00
48. Wyo.	116	68	563	1558	212	701	859	1171	6.20

TABLE 2—(continued)

	95 percentile rental divided by 5 percentile rental, non-farm homes.	Membership 13 or more, all churches, per 10,000.	Membership 13 or more, Roman Catholic, per 10,000.	Membership 13 or more, Jewish Congregations, per 10,000.	Membership 13 or more, Unitarians, Universalists, etc., per million.	Males, per 1000 females.	Births per year per 1000.	Births per year per 1000 females aged 20-44.	Deaths in first year per 1000 live births.
	117	124	125	126	127	128	129	130	131
1. Ala.	9.7	3284	109	37	333	988	24.0	130	68
2. Ariz.	9.3	3264	1559	34	370	1132	23.7	130	118
3. Ark.	9.3	3252	94	27	251	1028	22.1	123	55
4. Calif.	5.6	2284	1122	270	4242	1076	14.7	74	38
5. Colo.	8.1	2483	848	192	3439	1051	18.1	99	31
6. Conn.	7.3	4289	2617	603	1871	905	17.2	89	57
7. Del.	8.0	3564	1189	217	0	1035	18.7	102	75
8. Fla.	10.6	3260	220	98	1859	1010	18.2	91	66
9. Ga.	10.5	2966	45	63	464	973	20.9	111	73
10. Idaho	6.7	2804	370	—	1759	1143	20.6	124	53
11. Ill.	8.2	3116	1203	482	3286	1031	16.7	82	59
12. Ind.	7.4	3422	701	77	2198	1026	18.3	100	60
13. Iowa	6.3	3394	841	52	2000	1032	17.3	96	52
14. Kan.	7.1	3132	665	27	1819	1045	17.9	99	54
15. Ky.	10.6	3008	406	62	514	1024	22.6	131	66
16. La.	10.2	3430	1933	71	404	994	20.3	105	72
17. Me.	7.8	2726	1486	97	9819	1013	20.3	120	73
18. Md.	7.8	3372	1045	454	579	1013	18.5	87	77
19. Mass.	5.9	4231	2891	526	12127	951	17.3	88	59
20. Mich.	6.9	2918	1380	195	1871	1084	20.4	108	62
21. Minn.	5.7	3610	1316	161	2940	1055	18.5	99	51
22. Miss.	8.8	2384	95	15	264	1000	23.9	129	66
23. Mo.	8.6	3309	1175	218	2022	1009	17.1	88	61
24. Mont.	7.5	1971	884	12	1705	1200	18.5	109	61
25. Neb.	6.1	3117	817	92	1560	1052	19.6	106	50
26. Nev.	10.0	1671	720	—	2137	1403	14.6	86	69
27. N. H.	6.3	3469	2055	47	9138	992	17.9	102	64
28. N. J.	6.6	3644	2097	610	1195	1010	16.8	84	57
29. N. M.	8.6	3637	2844	9	582	1074	28.5	166	134
30. N. Y.	6.6	3176	1954	1653	2061	1006	17.1	81	59
31. N. C.	10.5	3715	17	13	355	988	24.1	136	77
32. N. D.	7.1	3089	983	24	619	1119	21.7	132	61
33. Ohio	6.8	3265	1079	268	2500	1023	17.7	93	62
34. Okla.	9.0	1870	151	19	1079	1061	17.7	97	60
35. Ore.	7.0	2025	454	138	4101	1100	14.1	76	46
36. Pa.	7.4	3866	1516	429	862	1012	19.6	105	66
37. R. I.	6.5	4914	3634	372	3729	952	17.7	91	64
38. S. C.	9.3	3992	36	23	163	963	23.3	132	85
39. S. D.	6.3	3169	1005	6	794	1105	—	—	54
40. Tenn.	10.1	3027	75	77	534	994	20.1	108	73
41. Tex.	9.3	2910	650	75	743	1038	—	—	74
42. Utah	6.9	5485	229	43	1255	1049	25.4	149	55
43. Vt.	7.4	3463	1766	40	7725	1039	19.3	117	63
44. Va.	11.0	3433	106	96	366	1003	22.6	127	75
45. Wash.	6.4	1969	589	89	3619	1121	14.7	79	47
46. W. V.	10.5	2457	308	31	299	1060	24.0	140	74
47. Wis.	6.2	3696	1570	114	1832	1058	19.3	106	56
48. Wyo.	7.4	2226	636	—	1710	1238	19.3	111	65

TABLE 2—(continued)

	General death rate.	Deaths per year from syphilis, per million.	Deaths per year from typhoid, per million.	Deaths per year from diphtheria, per million.	Deaths per year from appendicitis, per million.	Deaths per year from puerperal diseases per million.	Median size of family in 1930.	Percentage of native-born whites.	Percentage of foreign-born whites.
	132	133	134	135	136	137	140	143	144
1. Ala.	107	154	69	101	120	204	3.81	61.1	1.2
2. Ariz.	140	90	83	80	131	157	3.29	60.0	6.6
3. Ark.	92	79	157	86	153	169	3.61	70.7	1.2
4. Calif.	116	113	17	199	137	79	2.77	70.5	21.6
5. Colo.	120	73	52	153	241	136	3.17	78.7	15.5
6. Conn.	105	63	8	246	121	80	3.45	55.7	42.4
7. Del.	135	117	46	236	111	120	3.28	75.0	11.9
8. Fla.	124	290	59	145	143	192	3.13	63.6	7.0
9. Ga.	112	139	140	115	117	199	3.75	60.8	0.9
10. Idaho	93	40	39	148	207	114	3.47	85.1	13.3
11. Ill.	109	63	18	246	172	90	3.30	68.1	27.5
12. Ind.	119	70	34	217	158	108	3.23	89.2	7.1
13. Iowa	105	51	17	217	173	90	3.28	86.1	13.0
14. Kan.	105	68	23	218	176	115	3.26	88.2	7.4
15. Ky.	109	70	139	135	138	135	3.66	88.3	1.7
16. La.	111	259	121	126	149	189	3.61	57.0	3.4
17. Me.	136	47	31	265	161	144	3.24	80.2	19.5
18. Md.	127	150	45	227	115	103	3.47	73.3	10.8
19. Mass.	117	39	8	251	145	108	3.43	56.8	41.9
20. Mich.	103	78	15	202	162	120	3.37	67.8	28.8
21. Minn.	100	55	7	208	161	90	3.52	68.8	30.3
22. Miss.	112	177	102	104	131	191	3.56	46.0	0.7
23. Mo.	119	79	62	217	165	113	3.20	86.1	7.4
24. Mont.	101	76	33	163	226	127	3.16	71.8	25.1
25. Neb.	96	49	16	224	198	106	3.40	81.5	16.9
26. Nev.	131	121	39	152	311	117	2.50	69.7	21.1
27. N. H.	134	26	15	267	158	114	3.12	71.6	28.2
28. N. J.	105	70	12	252	142	90	3.47	58.5	36.4
29. N. M.	147	120	106	80	174	246	3.67	76.5	3.8
30. N. Y.	116	88	12	287	158	96	3.31	55.6	41.2
31. N. C.	103	79	51	106	97	187	4.24	71.0	0.6
32. N. D.	81	20	20	176	177	110	3.94	63.0	35.7
33. Ohio	112	80	24	222	150	106	3.28	78.9	16.6
34. Okla.	84	58	106	90	160	123	3.60	87.3	2.4
35. Ore.	107	91	22	200	152	75	2.92	81.2	17.5
36. Pa.	111	69	20	243	126	116	3.61	71.1	24.3
37. R. I.	114	62	9	301	151	105	3.47	54.4	44.0
38. S. C.	116	153	160	109	86	239	4.10	53.3	0.6
39. S. D.	91	17	30	180	180	99	3.64	76.5	20.5
40. Tenn.	109	117	117	108	136	153	3.70	78.9	1.0
41. Tex.	99	101	102	120	154	144	3.53	71.6	3.4
42. Utah	93	28	25	152	245	113	3.86	79.6	18.2
43. Vt.	129	21	14	267	178	124	3.23	81.8	18.1
44. Va.	120	139	61	149	110	153	3.82	71.4	2.0
45. Wash.	106	105	19	201	147	91	3.00	73.0	25.0
46. W. V.	100	97	112	124	134	134	3.95	87.0	6.0
47. Wis.	103	49	9	219	155	94	3.47	74.2	25.1
48. Wyo.	89	40	36	131	259	139	3.18	79.1	16.2

TABLE 2—(continued)

	Percentage of boys gainfully employed.	Percentage of girls gainfully employed.	Married women employed, per 10,000.	Wage earners in factories, per 10,000.	Clergy, M., per million	Clergy, F., per 10 million.	Physicians, M., per million.	Physicians, F., per 10 million.	Nurses, M., per million.
	153	154	155	156	162	163	164	165	166
1. Ala.	22.6	12.3	321	454	1264	129	813	45	20
2. Ariz.	4.2	2.1	282	241	999	252	1084	184	37
3. Ark.	17.1	7.3	265	238	1219	221	1081	156	11
4. Calif.	1.3	0.4	351	512	987	642	1565	1540	118
5. Colo.	4.8	1.4	250	316	1245	502	1554	838	41
6. Conn.	3.2	2.8	255	1577	963	162	1201	510	35
7. Del.	2.3	1.4	266	981	1443	168	1170	236	12
8. Fla.	10.2	3.9	394	442	2018	558	1202	306	40
9. Ga.	20.6	8.8	390	544	1354	132	962	148	15
10. Idaho	3.1	0.4	171	352	975	652	843	180	22
11. Ill.	2.0	0.9	250	901	1062	262	1398	918	54
12. Ind.	2.2	0.4	222	970	1342	510	1208	466	28
13. Iowa	4.1	0.6	163	335	1456	365	1213	456	17
14. Kan.	3.2	0.6	185	250	1648	569	1123	394	23
15. Ky.	9.3	1.6	160	291	1221	218	1065	229	26
16. La.	13.5	6.6	308	417	1186	152	943	228	9
17. Me.	1.6	0.7	267	873	1174	502	1141	414	43
18. Md.	4.7	2.5	287	805	1369	215	1468	613	89
19. Mass.	2.3	2.0	262	1315	967	181	1375	1001	88
20. Mich.	1.9	0.6	236	1094	906	271	1090	426	51
21. Minn.	2.9	0.7	152	399	1291	191	1187	647	60
22. Miss.	30.7	18.8	464	259	1283	115	760	100	5
23. Mo.	5.8	1.5	229	555	1362	370	1473	466	47
24. Mont.	3.3	0.6	181	254	1036	279	902	260	28
25. Neb.	3.4	0.5	171	205	1599	335	1244	508	25
26. Nev.	3.0	0.4	262	242	835	330	1548	439	0
27. N. H.	1.4	1.2	329	1399	1326	473	1002	774	32
28. N. J.	2.2	2.4	250	1080	986	104	1079	494	49
29. N. M.	5.0	1.9	178	106	1070	94	872	260	26
30. N. Y.	1.9	1.3	259	875	987	141	1502	857	75
31. N. C.	15.1	7.2	294	655	1302	186	721	120	9
32. N. D.	4.6	0.9	107	59	1463	220	726	206	7
33. Ohio	1.7	0.4	229	1106	1103	313	1207	540	38
34. Okla.	5.5	1.2	192	130	1318	438	1032	296	8
35. Ore.	3.9	0.3	311	687	1279	828	1292	975	44
36. Pa.	2.0	2.1	173	1047	1143	156	1210	641	50
37. R. I.	3.1	3.0	286	1816	924	175	1091	451	71
38. S. C.	23.0	13.6	441	624	1410	104	715	132	12
39. S. D.	4.0	0.6	134	94	1631	289	838	260	9
40. Tenn.	12.2	3.5	248	485	1322	183	1136	191	32
41. Tex.	10.1	4.4	243	226	1394	261	1069	308	25
42. Utah	3.1	0.5	136	308	413	118	967	315	22
43. Vt.	2.6	1.0	219	767	1304	222	1251	612	39
44. Va.	7.3	2.0	243	489	1383	132	987	169	21
45. Wash.	2.6	0.6	276	733	1151	608	1201	698	33
46. W. V.	2.7	0.8	139	488	1144	208	998	225	24
47. Wis.	3.2	0.7	162	898	1126	136	993	318	21
48. Wyo.	3.7	1.0	214	279	856	399	944	399	0

TABLE 2—(continued)

	Trained nurses, F., per 100,000.	Domestic servants, M., per 100,000.	Domestic servants, F., per 100,000.	Teachers, M., per million.	Teachers, F., per 100,000.	Musicians, M., per million.	Musicians, F., per million.	Dentists, M., per million.	Dentists, F., per 10 million.
	167	168	169	171	172	173	174	177	178
1. Ala.	100	176	1595	1156	546	147	338	242	19
2. Ariz.	243	417	950	1869	762	677	562	338	46
3. Ark.	85	143	960	1702	501	194	376	208	16
4. Calif.	398	720	1088	1673	733	1517	1381	938	275
5. Colo.	278	303	1046	1843	910	724	987	727	68
6. Conn.	398	310	1337	1280	747	800	633	586	75
7. Del.	213	321	1848	1099	613	394	524	420	84
8. Fla.	189	442	2353	1149	652	665	654	466	27
9. Ga.	133	203	2062	959	590	180	394	312	34
10. Idaho	165	219	626	2656	892	440	658	535	67
11. Ill.	253	304	1280	1449	639	910	792	769	143
12. Ind.	171	156	963	2303	624	454	661	574	71
13. Iowa	227	125	1011	1512	1064	473	669	698	36
14. Kan.	184	174	895	2237	979	452	644	563	53
15. Ky.	104	169	972	1551	521	257	394	325	38
16. La.	138	229	2046	979	570	415	339	361	86
17. Me.	282	229	1262	1496	826	461	742	520	83
18. Md.	310	348	2077	1236	575	651	484	503	13
19. Mass.	423	362	1420	1562	763	948	900	793	160
20. Mich.	236	232	1108	1442	674	637	597	509	75
21. Minn.	328	215	1314	1424	910	622	721	757	113
22. Miss.	80	161	1380	1271	559	124	259	216	0
23. Mo.	157	283	1204	1648	647	637	630	670	102
24. Mont.	253	287	877	1787	1054	472	644	534	56
25. Neb.	220	165	967	1708	1081	574	748	708	58
26. Nev.	221	805	918	2120	813	944	725	670	329
27. N. H.	357	249	1245	1571	812	522	720	481	150
28. N. J.	262	353	1408	1254	696	880	605	577	106
29. N. M.	120	278	911	2223	739	361	383	234	24
30. N. Y.	330	513	1663	1365	687	1557	826	747	239
31. N. C.	139	207	1390	1269	639	159	292	242	32
32. N. D.	211	123	1005	2409	1190	308	357	452	29
33. Ohio	230	207	1193	1699	633	665	707	577	95
34. Okla.	112	220	841	2205	704	363	456	329	71
35. Ore.	288	407	1048	1749	874	795	1367	911	147
36. Pa.	259	212	1325	1530	630	656	507	602	118
37. R. I.	290	317	1212	1048	664	258	800	543	116
38. S. C.	118	188	1762	1130	656	88	299	224	0
39. S. D.	185	98	871	2209	1195	401	488	498	14
40. Tenn.	133	242	1467	1514	572	265	438	309	50
41. Tex.	137	294	1226	1678	683	454	567	336	60
42. Utah	191	223	661	3064	718	768	705	640	20
43. Vt.	294	184	1520	1084	884	345	712	414	139
44. Va.	190	260	1677	1028	672	237	871	320	37
45. Wash.	290	464	987	1903	755	915	1117	854	179
46. W. V.	142	147	895	2733	675	216	318	387	23
47. Wis.	213	141	1056	1564	741	510	524	722	82
48. Wyo.	195	350	818	2199	1086	372	603	479	0

TABLE 2—(continued)

	Female/male ratio for professional workers except teachers.	Ratio of dentists to lawyers (male).	Residents listed in Who's Who for 1930, per million.	Residents listed in Who's Who for 1910, per million.	Income-tax returns of \$5,000 or over per 1000.	Membership in the A.A.A.S. per million.	Average salary of full-time workers in all retail stores.	Average yearly wage of workers in manufacturing plants.	Number of income-tax returns, per 1000.
	207c	207d	208	209	235	211	220	223	233
1. Ala.	.38	.40	105	44	1.9	34	\$1071	\$ 792	8
2. Ariz.	.38	.27	253	76	3.4	248	1341	1351	22
3. Ark.	.33	.28	74	33	1.6	23	1073	825	5
4. Calif.	.51	.54	313	103	10.7	257	1455	1491	60
5. Colo.	.50	.49	315	192	4.7	179	1249	1262	27
6. Conn.	.55	.51	539	319	8.4	243	1429	1172	44
7. Del.	.86	.49	315	197	6.3	302	1218	1165	38
8. Fla.	.34	.27	174	46	6.3	96	1121	764	19
9. Ga.	.41	.33	133	47	2.0	40	1020	664	10
10. Idaho	.42	.41	180	76	2.1	88	1309	1318	15
11. Ill.	.42	.51	261	152	9.6	149	1438	1351	40
12. Ind.	.40	.50	138	83	3.8	91	1206	1212	19
13. Iowa	.50	.65	149	79	3.0	119	1164	1183	15
14. Kan.	.45	.59	138	85	3.8	94	1165	1275	17
15. Ky.	.33	.33	118	68	2.8	49	1103	1054	11
16. La.	.38	.47	92	55	3.7	90	1022	902	15
17. Me.	.59	.56	285	222	5.0	104	1174	1019	22
18. Md.	.40	.30	354	196	3.8	274	1170	1068	41
19. Mass.	.58	.51	488	416	9.1	254	1315	1169	48
20. Mich.	.42	.56	122	60	5.4	112	1459	1406	29
21. Minn.	.57	.63	199	94	3.4	134	1228	1216	21
22. Miss.	.37	.35	60	31	1.1	22	1017	732	6
23. Mo.	.39	.45	195	105	5.3	112	1200	1123	22
24. Mont.	.48	.41	203	86	2.4	121	1398	1476	19
25. Neb.	.49	.56	182	105	3.7	103	1193	1268	19
26. Nev.	.32	.27	494	187	5.8	329	1593	1621	42
27. N. H.	.64	.63	417	337	5.7	189	1177	1027	29
28. N. J.	.34	.36	251	158	10.6	165	1483	1291	46
29. N. M.	.33	.29	248	73	1.8	142	1159	1251	15
30. N. Y.	.45	.35	463	311	12.8	264	1506	1398	55
31. N. C.	.44	.33	128	44	1.8	57	1116	716	8
32. N. D.	.50	.52	125	60	1.4	69	1203	1402	12
33. Ohio	.41	.44	171	97	6.1	134	1341	1329	28
34. Okla.	.34	.23	113	28	2.7	55	1212	1234	13
35. Ore.	.54	.55	195	70	5.5	128	1308	1225	23
36. Pa.	.41	.73	194	132	7.1	136	1257	1223	31
37. R. I.	.51	.56	342	242	7.6	163	1281	1075	35
38. S. C.	.45	.35	119	45	1.2	40	1019	629	7
39. S. D.	.43	.47	182	69	1.6	42	1181	1216	13
40. Tenn.	.39	.33	158	72	2.8	63	1078	852	12
41. Tex.	.38	.30	89	29	3.7	67	1161	1096	18
42. Utah	.39	.54	213	102	4.0	144	1272	1213	22
43. Vt.	.60	.46	364	281	4.8	117	1197	1175	23
44. Va.	.43	.32	202	99	2.8	111	1136	998	15
45. Wash.	.51	.59	196	76	5.8	100	1369	1290	36
46. W. V.	.34	.43	120	40	3.1	60	1259	1247	15
47. Wis.	.43	.83	145	79	4.2	119	1311	1150	32
48. Wyo.	.41	.37	275	129	4.6	102	1400	1639	32

TABLE 2—(continued)

	Y. M. C. A., membership, per 1000.	Y. M. C. A., net property value, per 1000.	Y. M. C. A., annual expenditures, per 1000.	Deaths from homicide per year, per million.	Deaths from suicide, per year, per million.	Deaths from automobile accidents per year, per million.	Membership in the Boy Scouts, per 10,000.	Membership in Kiwanis, per 100,000.	Membership in Rotary, per 100,000.	Percentage of total public expenditures spent for libraries.
	Used to compute 248			241	242	243	249	251	252	261
1. Ala.	5.9	\$ 601	\$ 147	217	74	183	29.2	53	46	.01
2. Ariz.	5.2	959	345	135	165	362	74.4	103	173	.03
3. Ark.	1.4	49	50	157	65	158	25.1	31	94	.01
4. Calif.	8.7	1548	425	71	273	405	188.9	117	141	.02
5. Colo.	9.4	1101	499	78	205	282	57.8	88	126	.00
6. Conn.	18.8	4805	851	27	170	275	92.8	58	109	.07
7. Del.	12.1	5592	1027	88	148	327	38.7	92	150	.02
8. Fla.	2.3	797	148	271	146	353	108.2	140	144	.00
9. Ga.	6.8	703	144	199	101	206	16.9	81	40	.01
10. Idaho	5.5	457	195	39	153	224	126.9	156	174	.01
11. Ill.	8.0	2533	799	102	172	278	103.7	67	83	.03
12. Ind.	12.0	1690	551	66	191	340	74.2	186	108	.03
13. Iowa	7.6	1078	303	28	196	212	59.1	83	136	.05
14. Kan.	6.6	841	256	63	157	260	67.4	91	151	.03
15. Ky.	5.9	710	328	186	113	180	27.7	52	74	.01
16. La.	2.5	443	98	194	89	184	35.9	30	53	.02
17. Ma.	6.2	1904	306	19	150	207	74.1	117	180	.04
18. Md.	7.3	1041	398	84	168	268	37.2	34	68	.02
19. Mass.	12.6	2872	853	20	135	188	83.3	76	101	.02
20. Mich.	7.8	2056	526	64	167	292	97.2	63	91	.01
21. Minn.	6.1	1183	418	30	162	222	74.0	81	58	.02
22. Miss.	4.2	681	92	227	53	149	26.4	38	88	.01
23. Mo.	5.2	1209	343	112	182	215	45.0	50	63	.01
24. Mont.	10.0	1775	287	87	225	221	75.7	195	161	.01
25. Neb.	6.9	872	340	34	177	208	66.2	84	119	.02
26. Nev.	0	0	0	201	410	569	82.1	134	304	.02
27. N. H.	15.8	1765	606	15	157	221	41.3	82	241	.03
28. N. J.	12.9	2822	693	50	164	297	107.7	87	93	.02
29. N. M.	2.1	331	68	135	124	258	63.2	99	100	.00
30. N. Y.	10.1	2671	684	57	184	240	104.4	41	71	.01
31. N. C.	6.9	838	160	113	82	209	27.5	86	77	.02
32. N. D.	2.9	498	127	17	101	140	56.8	114	77	.01
33. Ohio	14.2	2145	844	85	174	322	66.8	121	98	.01
34. Okla.	4.2	415	137	111	97	193	60.4	102	124	.01
35. Ore.	9.0	1552	532	44	234	270	93.7	160	116	.03
36. Pa.	12.2	2276	537	50	145	241	76.8	73	90	.01
37. R. I.	9.5	2313	533	21	117	133	74.0	23	43	.04
38. S. C.	6.0	367	115	146	66	202	31.3	47	56	.01
39. S. D.	4.5	888	202	19	118	168	56.1	137	87	.02
40. Tenn.	4.2	685	184	102	106	195	51.0	53	62	.01
41. Tex.	4.0	562	149	166	124	231	63.9	58	120	.00
42. Utah	0	0	0	44	104	276	228.3	117	105	.00
43. Vt.	4.5	884	246	14	163	198	77.3	4	253	.03
44. Va.	8.7	1009	343	125	123	233	51.0	85	97	.02
45. Wash.	10.6	1426	469	51	247	294	87.3	191	135	.01
46. W. V.	4.9	542	307	125	109	212	76.2	110	108	.01
47. Wis.	7.1	1094	387	26	174	247	70.1	116	108	.05
48. Wyo.	1.4	0	17	92	223	355	129.0	84	128	.02

Composite Scores

For each city and each state three weighted composite scores G, I, and P have been computed which may be used as indices respectively of the general goodness of life for good people in the community in question, the per capita income of its residents, and their personal qualities of intelligence, morality and care for their families. These G, I, and P scores are based upon 37, 10, and 11 items respectively, as listed below.

CONSTITUENTS OF THE G SCORE

Item	ITEMS OF HEALTH	Approximate Weight for	
		Cities	States
131.	Infant death-rate reversed	12	13
132.	General death-rate reversed	9½	9
134.	Typhoid death-rate reversed	5	5½
136.	Appendicitis death-rate reversed	4	3½
137.	Puerperal diseases death-rate reversed	4	5½

ITEMS OF EDUCATION

53.	Per capita public expenditures for schools	8	8
54.	Per capita public expenditures for teachers' salaries	6	7
55.	Per capita public expenditures for textbooks and supplies	7	8
56.	Per capita public expenditures for libraries and museums	6½	6
21.	Percentage of persons sixteen to seventeen attending schools	4½	4¼
22.	Percentage of persons eighteen to twenty attending schools	7	7½
23.	Average salary, high-school teacher	3½	7½
24.	Average salary, elementary-school teacher	3½	

ITEMS OF RECREATION

57.	Per capita public expenditures for recreation	7	7½
17.	Per capita acreage of public parks	2½	2

ECONOMIC AND "SOCIAL" ITEMS

107.	Rarity of extreme poverty	6	6¼
108.	Rarity of less extreme poverty	6	6½
153.	Infrequency of gainful employment for boys 10-14	5	4½
154.	Infrequency of gainful employment for girls 10-14	5½	5½
223.	Average wage of workers in factories	4	4
106.	Frequency of home ownership (per capita number of homes owned)	6	6
248.	Per capita support of the Y. M. C. A.	6	6
201.	Excess of physicians, nurses, and teachers over male domestic servants	6	6

Item	CREATURE COMFORTS	Approximate Weight for	
		Cities	States
98.	Per capita domestic installations of electricity.....	5	5½
99.	Per capita domestic installations of gas.	7	7
102.	Per capita number of automobiles.....	4	5
103.	Per capita domestic installations of telephones.....	11	10
104.	Per capita domestic installations of radios.....	6½	6½

OTHER ITEMS

31.	Percentage of literacy in the total population.....	3½	4
25.	Per capita circulation of Better Homes and Gardens, Good Housekeeping and the National Geographic Magazine..	6	6
26.	Per capita circulation of the Literary Digest.....	6	5½
133.	Death rate from syphilis (reversed).....	4	4
241.	Death rate from homicide (reversed).....	3½	3½
243.	Death rate from automobile accidents (reversed).....	4½	3½
12.	Per capita value of asylums, schools, libraries, museums, and parks owned by the public.....	6	6
16.	Ratio of value of schools, etc., to value of jails, etc.....	10*	3
11.	Per capita public property minus public debt.....	5	5

After the 37 scores were multiplied by amounts such as to make their standard deviations be proportional to the numbers listed above as approximate weights, the sum for each city (called G3) was combined with a score (G1) which was computed by subtracting the number of features among the 37 in which that city was below the median of the 295 cities from the number in which it was above the median of the 295.† G3 and G1 had relative weights of approximately 2 and 1 respectively in the final G score.

In the states the constituents of G were the same except that items 23 and 24 were replaced by item 24a, the average salary of all teachers and supervisors. Item 24a was given approximately as much weight in states as items 23 and 24 together had in cities.

It should be kept in mind that Items 11, 12, 16, 56, and 57 are for a state's own property, debt, and expenditures, not for these plus those of the smaller governmental units within its boundaries. These items

* The weight given item 16 is much too high, but the harm done is inappreciable. The error, of multiplying by 2 instead of dividing by 2, was discovered only after most of the correlations had been computed. Changes in them would be inappreciable, since the correlation between the G in which Item 16 has a weight of only 2½ and the G as used is almost 1.00.

† In computing the standard deviations of the constituents of G, I, and P, the influence of extreme and possibly erroneous measures was minimized by taking as the standard deviation, one half the distance along the scale required to exclude 47 cities or 8 states at each extreme.

In the case of four very extreme state scores (expenses of recreation for Connecticut and for Wyoming; park acreage for Connecticut and park acreage for Arizona), I have reduced the weight in the composite to half what it would be if the scores were taken at their face value.

are consequently not so significant for welfare as the corresponding items for cities. Item 53 for states does include all governmental units, but is the per capita expenditures for schools including expenditures for building (average of 1930 and 1932 from p. 114 of the Statistical Abstract of the United States for 1935). The figures which would be strictly comparable with item 53 in cities are not available for states.

CONSTITUENTS OF THE I SCORE

After the investigation reported in the Journal of the American Statistical Association (Sept. '37, vol. 32, pp. 471-479) and some further work, the following items and weights were chosen for final use:

	Standard Deviation	Multi- plier	Weight	Approx. Relative Weights
Income-tax returns (over \$2,500).....	12.7	3	38.1	15
Income-tax returns (over \$5,000).....	5.8	3	17.4	7
Average wages: teachers.....	7.6	1	7.6	3
Average wages: retail store employees....	13.5	1	13.5	5
Average wages: factory employees.....	8.3	2	16.6	6
Expenditures: rent (or equivalent).....	3.95	2	7.9	3
Expenditures: food store sales.....	5.9	2	11.8	4
Expenditures: cigar store sales.....	5.3	$\frac{1}{2}$	2.65	1
Expenditures: drug store sales.....	5.7	$\frac{1}{2}$	2.85	1

The resulting I score or index for each city is shown in TABLE 3. That it is positively and closely correlated with the real incomes is obvious from the cities which stand at the top and bottom of the list. They are:

The Ten Highest Cities in I		The Ten Lowest Cities in I	
Evanston, Ill.....	35	Pensacola, Fla.....	-11
Oak Park, Ill.....	39	Augusta, Ga.....	-14
Brookline, Mass.....	36	Columbus, Ga.....	-15
Newton, Mass.....	24	Macon, Ga.....	-13
Montclair, N. J.....	36	Paducah, Ky.....	-11
Plainfield, N. J.....	24	Meridian, Miss.....	-13
Mt. Vernon, N. Y.....	30	Wilmington, N. C.....	-11
New Rochelle, N. Y.....	30	Charleston, S. C.....	-13
White Plains, N. Y.....	36	Laredo, Tex.....	-19
Cleveland Hts., O.....	30	Portsmouth, Va.....	-11

The first five items are directly related to income and represent levels of it from high to low. The last four are measures of expenditures, and so, indirectly, of income. They were chosen to represent the expenditures of residents rather than non-residents, and of a fair sampling of all income levels. The list has one notable weakness, in

that the expenditures are such as respectable people make for respectable purposes. The expenditures for prostitutes, gambling, forbidden drugs, intoxicants, and more or less disreputable entertainment in these cities could not be estimated. This weakness acts as a factor of safety in the case of some of our most important conclusions.

The standard deviations, multipliers and consequent weights used for the states were as follows:

	Vari- ation	Multi- plier	Weight	Approx. Weights
Income tax returns (over \$2500).....	4.5	15	67.5	15
Income tax returns (over \$5000).....	2.75	10	27.5	7
Average wages: teachers and supervisors..	3.75	4	15.0	3½
Average wages: retail store employees....	13.75	2	27.5	7
Average wages: factory employees.....	9.5	3	28.5	7
Expenditures: rent (or equivalent)....	3.75	4	15.0	3½
Expenditures: food-store sales.....	6.25	3	18.75	4½
Expenditures: cigar-store sales.....	4.5	1	4.5	1
Expenditures: drug-store sales.....	4.0	1	4.0	1

CONSTITUENTS OF THE P SCORE

The personal qualities index, P, is a weighted composite of the deviations from the median in the items listed below, the weights being approximately as stated.

Item	Standard Deviation	Multi- plier	Weight	Approx. Weights
30. Per capita number of graduates from public high schools in 1934.....	4.5	4	18.0	4½
261. Percentage which public expenditures for the maintenance of libraries was of the total.....	4.2	2	8.4	2
31. Percentage of illiteracy (reversed)...	10.3	1	10.3	2½
33. Percentage of illiteracy among those aged 15-24 (reversed).....	11.4	1	11.4	3
41. Per capita circulation of public libraries.....	3.2	6	19.2	5
106. Per capita number of homes owned.	3.0	6	18.0	4½
201. Per capita number of physicians, nurses and teachers minus male domestic servants.....	3.0	5	15.0	4
103. Per capita number of telephones....	3.8	3	11.4	3
207d. Number of male dentists divided by number of male lawyers.....	4.0	2	8.0	2
133. Per capita number of deaths from syphilis (reversed).....	6.1	2	12.2	3
241. Per capita number of deaths from homicide (reversed).....	12.0	1	12.0	3

The standard deviations, multipliers and consequent weights used for the states were:

Item	Variability	Multiplier	Weight	Approx. Weight
30.	$5\frac{1}{4}$	12	63	5
261.	1	25	25	2
31.	20	2	40	$2\frac{3}{4}$
33.	54	0.8	43	$2\frac{1}{2}$
41.	data not available			
106.	$2\frac{1}{2}$	25	63	5
201.	2	25	50	4
103.	$4\frac{1}{4}$	10	43	$2\frac{1}{2}$
207d.	$2\frac{1}{2}$	10	25	2
133.	$3\frac{1}{4}$	12	39	$2\frac{3}{4}$
241.	9	5	45	$2\frac{1}{2}$

G, I, AND P SCORES FOR EACH CITY AND EACH STATE

TABLE 3 presents the three scores (for general goodness of life, per capita income, and personal qualities) of each city. TABLE 4 presents similar, but not strictly comparable, scores for each state. The scores of TABLE 3 are deviations from the status of the median city. The scores of TABLE 4 are deviations from the status of the median state; and the relative weights of the items in the composites are not absolutely the same for states as for cities, though they are approximately so.

The comparison of the goodness of life in any state with that in any city would in any case be difficult because the conditions are different. The entries of TABLE 4 should not be compared with those of TABLE 3. It is however true that the lowest states in G are lower than the lowest cities in G, and that the highest states are not nearly so high as the highest cities.

Comparisons between one state and another are not so safe as comparisons between one city and another because the states differ more among themselves in the character of life, the cost of living and other respects, than cities of 30,000 to 500,000 population do.

The plus and minus G scores of TABLE 3 may be interpreted as follows: If a city had as low a score in each of the 37 traits as the lowest city in that trait had, it would score about - 38. If a city had as high a score in each of the 37 traits as the highest city in that trait had, it would score about + 46. The difference between the two would be about 84. The difference between the median city and Pasadena is three-tenths of this, and the difference between the median city and the lowest four or five is one-fourth of it. Consider a city in

which half of the babies born die within a year, no schooling or recreational facilities is furnished free to anybody, 98 percent of the population live in mud hovels, eating food costing less than 10 cents per day and owning nothing but a few rags; in which one person in five hundred is murdered every year and another dies from typhoid; in which ninety per cent of boys and girls ten to fourteen are at work and nine out of ten teachers are slaves; in which the status in all the other items is correspondingly low. Such a city would score about -140 by our system. Calling its welfare zero, the welfare of our lowest cities is about 120, and that of our highest is about 165.

The plus and minus I scores of TABLE 3 may be interpreted as approximately the number of dollars stated below:

I Score	Estimated Approximate Annual Income per Resident including all ages, in 1930*
-20	\$200
-10	300
0	450
+10	700
+20	1000
+30	1300

The plus and minus P scores of TABLE 3 may be left for the present as arbitrary values.

TABLE 3
SCORES OF CITIES IN G, I, AND P
0 = the median city

	General Good- ness G	In- come I	Per- sonal Qual- ities P		General Good- ness G	In- come I	Per- sonal Qual- ities P
ALABAMA				CALIFORNIA			
Birmingham....	-13	- 5	-19	Alameda.....	14	6	15
Mobile.....	-16	- 8	-22	Berkeley.....	19	9	13
Montgomery...	-18	- 9	-25	Fresno.....	9	6	6
ARIZONA				Glendale.....	17	12	20
Phoenix.....	- 3	10	- 2	Long Beach...	15	10	7
Tucson.....	- 9	0	- 5	Oakland.....	13	10	6
ARKANSAS.....				Pasadena.....	24	20	27
Fort Smith.....	- 8	-11	- 3	Sacramento...	7	7	- 4
Little Rock.....	-14	- 4	-14	San Bernardino.	- 2	- 1	- 2
				San Diego.....	11	4	5
				San Jose.....	13	6	7

* I use a composite of the estimates of income for these cities made by commercial concerns for the use of advertisers.

TABLE 3—(continued)

	General Good- ness G	In- come I	Per- sonal Quali- ties P		General Good- ness G	In- come I	Per- sonal Quali- ties P
CALIFORNIA—Cont.				ILLINOIS—Cont.			
Santa Ana.....	12	7	8	Quincy.....	- 5	- 5	5
Santa Barbara..	17	21	14	Rockford.....	6	4	8
Santa Monica..	16	10	9	Rock Island....	3	- 7	7
Stockton.....	8	8	12	Springfield....	- 2	0	5
				Waukegan.....	7	11	6
COLORADO				INDIANA			
Colorado Springs	12	7	15	Anderson.....	1	- 9	6
Denver.....	6	4	4	E. Chicago.....	- 6	- 1	-15
Pueblo.....	- 1	- 9	- 1	Elkhart.....	5	- 1	10
CONNECTICUT				Evansville.....	- 5	- 7	0
Bridgeport.....	1	1	- 1	Fort Wayne....	6	1	7
Hartford.....	7	10	- 4	Gary.....	- 2	6	- 9
Meriden.....	0	2	3	Hammond.....	1	- 2	1
New Britain....	1	1	- 9	Indianapolis...	- 1	1	4
New Haven....	4	4	0	Kokomo.....	- 3	- 9	0
Norwalk.....	- 4	6	- 2	Muncie.....	- 2	- 3	5
Stamford.....	5	14	1	Richmond.....	4	- 3	4
Waterbury.....	1	2	- 4	South Bend....	3	1	3
				Terre Haute....	1	- 3	4
DELAWARE				IOWA			
Wilmington....	- 1	6	- 6	Cedar Rapids...	7	- 3	13
DISTRICT OF COLUMBIA				Council Bluffs..	1	- 4	11
FLORIDA				Davenport.....	4	- 1	8
Jacksonville....	-12	- 6	-26	Des Moines.....	7	0	9
Pensacola.....	-16	-11	-17	Dubuque.....	0	- 5	15
Tampa.....	- 7	- 8	-16	Sioux City.....	0	- 2	5
				Waterloo.....	4	- 4	8
GEORGIA				KANSAS			
Atlanta.....	-13	- 3	-21	Kansas City....	- 8	- 8	- 6
Augusta.....	-21	-14	-32	Topeka.....	6	0	5
Columbus.....	-21	-15	-27	Wichita.....	3	- 2	5
Macon.....	-18	-13	-23	KENTUCKY			
Savannah.....	-19	- 9	-31	Covington.....	- 7	3	- 9
ILLINOIS				Lexington.....	-13	2	-20
Alton.....	- 4	- 3	- 1	Louisville.....	- 9	- 2	- 7
Aurora.....	5	4	11	Paducah.....	-15	-11	-13
Berwyn.....	7	10	6	LOUISIANA			
Bloomington....	1	2	9	Baton Rouge...	- 9	- 5	-10
Cicero.....	2	4	2	New Orleans....	-17	- 9	-26
Danville.....	- 3	- 2	7	Shreveport....	-16	- 3	-19
Decatur.....	- 2	- 3	6	MAINE			
East St. Louis..	- 8	- 3	- 9	Lewiston.....	-15	- 8	- 9
Elgin.....	2	3	6	Portland.....	1	1	2
Evanston.....	18	35	13	MARYLAND			
Joliet.....	5	8	10	Cumberland....	- 6	- 2	- 2
Moline.....	0	- 3	7	Hagerstown....	-12	- 8	-10
Oak Park.....	18	39	22				
Peoria.....	- 1	1	3				

TABLE 3—(continued)

	General Good- ness G	In- come I	Per- sonal Quali- ties P		General Good- ness G	In- come I	Per- sonal Quali- ties P
MASSACHUSETTS				MISSOURI			
Arlington.....	8	13	11	Joplin.....	- 4	- 7	5
Brockton.....	0	0	6	Kansas City....	0	3	- 3
Brookline.....	19	36	17	St. Joseph.....	- 9	- 7	- 1
Cambridge.....	5	7	- 1	Springfield.....	- 7	-10	5
Chelsea.....	- 5	- 4	-11	MONTANA			
Chicopee.....	- 9	- 7	- 4	Butte.....	- 1	7	1
Everett.....	4	0	- 2	NEBRASKA			
Fall River.....	- 7	-11	- 9	Lincoln.....	7	- 2	15
Fitchburg.....	- 2	- 4	1	Omaha.....	5	2	3
Haverhill.....	2	- 3	6	NEW HAMPSHIRE			
Holyoke.....	0	0	1	Manchester....	- 7	- 7	- 1
Lawrence.....	- 4	- 4	- 7	Nashua.....	- 7	- 4	1
Lowell.....	- 7	- 6	- 2	NEW JERSEY			
Lynn.....	5	2	1	Bayonne.....	0	3	- 8
Malden.....	5	0	4	Bloomfield.....	9	12	3
Medford.....	8	2	6	Camden.....	0	- 4	-15
New Bedford...	- 6	- 6	-10	Clifton.....	0	1	- 7
Newton.....	14	24	16	East Orange....	13	20	8
Pittsfield.....	3	3	4	Elizabeth.....	0	10	- 6
Quincy.....	8	8	10	Hoboken.....	- 1	7	-18
Revere.....	1	- 8	- 3	Irvington.....	5	8	- 4
Salem.....	- 3	1	- 1	Jersey City....	1	6	-10
Somerville.....	1	5	- 1	Kearney.....	3	- 1	- 4
Springfield.....	11	5	9	Montclair.....	20	36	7
Taunton.....	- 6	- 7	1	Newark.....	1	9	-11
Waltham.....	3	0	- 4	New Brunswick..	0	9	- 4
Watertown.....	6	4	7	Orange.....	4	15	- 5
Worcester.....	5	2	1	Passaic.....	- 2	10	- 8
MICHIGAN				Paterson.....	0	6	- 7
Battle Creek....	8	6	9	Perth Amboy... Plainfield.....	- 4 10	2 24	- 5 7
Bay City.....	- 2	- 7	5	Trenton.....	- 4	4	- 3
Dearborn.....	6	2	- 1	Union City.....	0	5	- 9
Flint.....	2	0	5	West New York	6	7	- 9
Grand Rapids... Hamtramck....	10 0	6 - 4	10 -12	NEW YORK			
Highland Park..	8	10	4	Albany.....	6	6	- 2
Jackson.....	6	0	4	Amsterdam....	- 2	- 3	- 3
Kalamazoo.....	10	7	12	Auburn.....	- 5	- 1	- 1
Lansing.....	5	2	4	Binghamton....	3	0	- 1
Muskegon.....	6	0	11	Elmira.....	6	3	7
Pontiac.....	1	3	- 5	Jamestown.....	6	4	1
Port Huron....	4	- 1	10	Mount Vernon..	12	30	4
Saginaw.....	- 1	- 0	6	Newburgh.....	5	2	- 1
MINNESOTA				New Rochelle... Niagara Falls..	14 5	30 2	1 - 2
Duluth.....	8	- 1	5	Poughkeepsie.. Rochester.....	2 9	5 7	- 2 2
Minneapolis... St. Paul.....	10 6	1 1	6 4				
MISSISSIPPI							
Jackson.....	-18	- 9	-29				
Meridian.....	-21	-13	-22				

TABLE 3—(continued)

	General Good- ness G	In- come I	Per- sonal Quali- ties P		General Good- ness G	In- come I	Per- sonal Quali- ties P
NEW YORK—Cont.				PENNSYLVANIA—Cont.			
Rome.....	- 8	- 6	-30	Bethlehem.....	- 1	- 5	- 4
Schenectady.....	7	8	4	Chester.....	- 8	1	-13
Syracuse.....	6	4	- 1	Easton.....	5	7	10
Troy.....	- 3	- 1	- 6	Erie.....	4	1	2
Utica.....	- 4	1	- 6	Harrisburg.....	5	2	4
Watertown.....	4	2	4	Hazleton.....	- 7	- 9	3
White Plains.....	17	36	10	Johnstown.....	- 1	0	0
Yonkers.....	6	16	- 6	Lancaster.....	0	1	2
NORTH CAROLINA				McKeesport.....	- 3	5	1
Asheville.....	- 7	- 4	- 9	New Castle.....	2	- 2	5
Charlotte.....	-14	- 2	-18	Norristown.....	- 5	7	- 6
Durham.....	-19	-10	-24	Reading.....	0	1	- 1
Greensboro.....	-12	- 5	- 9	Scranton.....	- 2	- 3	- 3
High Point.....	-20	-10	-17	Wilkes Barre....	0	- 1	1
Raleigh.....	-14	- 3	-17	Williamsport....	3	- 1	5
Wilmington.....	-17	-11	-21	York.....	- 1	- 1	1
Winston Salem..	-17	-10	-22	RHODE ISLAND			
OHIO				Cranston.....	- 4	- 4	- 2
Akron.....	- 1	- 3	- 4	Pawtucket.....	- 6	- 2	- 4
Canton.....	4	8	- 4	Providence.....	- 3	3	- 4
Cincinnati.....	1	10	- 6	Woonsocket.....	-11	- 3	- 9
Cleveland Heights	20	30	26	SOUTH CAROLINA			
Columbus.....	- 1	1	- 5	Charleston.....	-20	-13	-48
Dayton.....	3	3	1	Columbia.....	-18	- 9	-25
East Cleveland..	14	9	16	SOUTH DAKOTA			
Hamilton.....	- 2	- 4	0	Sioux Falls.....	6	- 2	6
Lakewood.....	15	23	12	TENNESSEE			
Lima.....	- 1	- 6	8	Chattanooga....	-16	- 7	-18
Lorain.....	0	- 3	- 0	Knoxville.....	-15	- 7	-10
Mansfield.....	7	3	6	Memphis.....	-14	- 4	-20
Marion.....	- 3	- 6	2	Nashville.....	-12	- 5	-14
Newark.....	1	- 6	5	TEXAS			
Norwood.....	6	6	2	Amarillo.....	- 6	5	- 6
Portsmouth.....	- 4	- 4	- 4	Austin.....	- 9	- 5	-16
Springfield.....	0	- 2	0	Beaumont.....	-11	- 5	-12
Steubenville.....	- 2	7	- 8	Dallas.....	- 8	2	-10
Toledo.....	5	4	0	El Paso.....	-12	- 8	-22
Warren.....	- 1	1	0	Fort Worth.....	- 7	- 4	-10
Youngstown.....	4	6	- 3	Galveston.....	-13	- 2	-20
Zanesville.....	- 4	- 3	3	Houston.....	- 9	1	-12
OKLAHOMA				Laredo.....	-18	-19	-32
Muskogee.....	- 4	- 5	5	Port Arthur.....	-11	- 6	-16
Oklahoma City..	- 6	- 2	- 5	San Antonio.....	-12	- 8	-25
Tulsa.....	2	6	- 2	Waco.....	- 8	- 6	- 3
OREGON				Wichita Falls...	- 1	2	- 6
Portland.....	7	1	7	UTAH			
PENNSYLVANIA				Ogden.....	- 2	- 5	9
Allentown.....	- 5	1	- 1	Salt Lake City..	5	- 1	9
Altoona.....	0	- 4	4				

TABLE 3—(continued)

	General Good- ness G	In- come I	Per- sonal Quali- ties P		General Good- ness G	In- come I	Per- sonal Quali- ties P
VIRGINIA				WEST VIRGINIA			
Lynchburg.....	-12	- 6	-14	Charleston.....	- 6	3	- 7
Newport News..	-12	- 9	-17	Huntington....	- 5	1	- 9
Norfolk.....	-12	- 5	-15	Wheeling.....	- 2	7	- 3
Portsmouth..	-13	-11	-20	WISCONSIN			
Richmond....	-11	- 1	-12	Green Bay.....	5	- 2	11
Roanoke.....	- 7	- 3	- 7	Kenosha.....	4	3	8
WASHINGTON				La Crosse.....	- 1	- 2	14
Bellingham.....	5	0	14	Madison.....	9	8	17
Everett.....	2	1	15	Milwaukee.....	6	3	0
Seattle.....	9	8	6	Oshkosh.....	5	- 4	14
Spokane.....	7	- 1	10	Racine.....	7	1	8
Tacoma.....	3	- 1	11	Sheboygan.....	2	- 2	9
				Superior.....	1	- 5	9
				West Allis.....	4	0	2

TABLE 4

SCORES OF STATES IN G, I, AND P

O = the median state

	G	I	P		G	I	P
1. Ala.....	-25	-15	-29	25. Neb.....	5	- 2	15
2. Ariz.....	-10	3	-14	26. Nev.....	2	16	- 6
3. Ark.....	-23	-15	-17	27. N. H.....	- 0	2	11
4. Cal.....	14	28	3	28. N. J.....	7	21	- 1
5. Col.....	- 2	4	4	29. N. M.....	-21	- 7	-19
6. Conn.....	11	16	8	30. N. Y.....	7	28	- 4
7. Del.....	1	8	- 4	31. N. C.....	-22	-15	-17
8. Fla.....	-22	- 5	-28	32. N. D.....	+ 0	- 6	9
9. Ga.....	-20	-15	-28	33. Ohio.....	6	8	3
10. Id.....	2	- 2	10	34. Okla.....	- 8	- 5	- 6
11. Ill.....	2	16	4	35. Ore.....	7	4	13
12. Ind.....	2	- 1	9	36. Pa.....	- 2	8	3
13. Iowa.....	5	- 4	19	37. R. I.....	- 1	8	- 1
14. Kan.....	3	- 4	14	38. S. C.....	-29	-17	-32
15. Ky.....	-19	-10	-14	39. S. D.....	+ 0	- 7	11
16. La.....	-24	-10	-29	40. Tenn.....	-22	-11	-17
17. Me.....	- 5	- 2	12	41. Tex.....	-17	- 5	-16
18. Md.....	-12	8	- 8	42. Utah.....	+ 0	+ 0	8
19. Mass.....	7	17	6	43. Vt.....	- 2	- 1	12
20. Mich.....	5	10	3	44. Va.....	-19	- 8	-15
21. Minn.....	7	- 0	11	45. Wash.....	10	10	9
22. Miss.....	-28	-17	-32	46. W. V.....	-13	- 4	- 9
23. Mo.....	- 7	+ 0	- 1	47. Wis.....	4	5	15
24. Mont.....	2	3	5	48. Wyo.....	5	7	5

CORRELATIONS

Intercorrelations of the Constituents of the G Score, for Cities

I have reported some of the most significant correlations in the case of the cities elsewhere.* I report here in TABLE 5 all the intercorrelations of the 37 constituents of the G score for the 295 cities and for the 200 cities remaining after the exclusion of cities adjoining larger cities and cities of the Old South. TABLE 5 proves that communities show a great specialization in these features of welfare, no city having them all in equal measure, and very few cities having them all in anything like equal measure. There is positive correlation as a rule, not compensation for inferiority in one respect by superiority in another. But the correlations are far from perfect. TABLE 6 shows that in the 200 cities over half of them are below .20. The wide range of the 295 cities raises the correlations somewhat above their value for the 200 cities, as shown in TABLE 6. The median correlation is .17 for the 200 cities and .32 for the 295 cities.

The variation among cities in goodness of life as measured by G is certainly not caused by any unitary mystical quality productive of all features of welfare uniformly. It is in fact caused largely (from three quarters to nine tenths of it) by two things—the intelligence, morality, family devotion, and other desirable personal qualities of the population and their income. But these operate in varied and complex ways. Two cities with equally high P scores and I scores will be closely alike in the total G score, but not in each of its features. One may spend on parks much of what the other spends on libraries and museums, or may take more care to keep boys and girls 16 and 17 in school and less care to keep children 10 to 14 out of gainful employment.

Intercorrelations of the Constituents of the G Score, for States

TABLE 5A presents the corresponding correlations for the states. The number of states is too small to permit argument from the exact amount of any of these correlations, but they are of value when taken in groups, and for cautious use singly in connection with the correlations for the 295 cities. The correlations are in general higher for the

* Your City, chapters IV and V, and *Amer. Jour. Sociology* 43: 191-224.

states, the median r being .42. They vary more widely. Deaths from automobile accidents show mostly negative correlations, being more frequent in the "good" states. This is presumably due to the much greater frequency of ownership of cars in these states. Deaths from appendicitis also show mostly negative correlations. This may be due in part to more accurate diagnosis in the better states.

Correlations of each Item with G, I, and P Scores, for Cities and for States

TABLE 7 presents six correlations for most of the items studied (with G, I, and P in the case of the 200 cities and the 295 cities), and three more correlations (with G, I and P in the case of the 48 states) for many of them. TABLE 7 may be used as a sociological test of the nature and merit of any feature of a community by its affiliations with general welfare for good people, wealth or income, and the personal qualities measured by the P score. Its use for partial correlations is specially important. If the feature in question has a positive partial correlation with G after equalization for P, there is hope that its benefits can be had by giving the community money to spend in the indicated direction. The hope is not assured, for the feature may be linked to G via personal qualities not fully represented in P, or may require that the I of the residents be earned by them, not received as a gift. If the feature has a positive partial correlation with G after equalization for I, there is hope that by it good people though poor may enhance the goodness of life in their community.

Intercorrelations of Certain Items

TABLE 8 presents some of the thousands of possible intercorrelations of single items and smaller composites than G, I, and P which are of special interest to students of human nature and human affairs. Such features of a community as its percentage of church members or of foreign-born are worth studying in respect of many of their affiliations. It is very seldom that science can by observation or experiment compare communities identical in all respects save one, and determine the consequences of that difference in and of itself. The social sciences must rely largely upon inferences concerning causation on the basis of affiliation, using the technique of partial and multiple correlation or some equivalent.

TABLE V

THE INTERCORRELATIONS OF THE MEASURES OF WHICH THE G SCORE IS A WEIGHTED COMPOSITE. THE NUMBERS UNDER 200 ARE CORRELATIONS FOR THE 200 CITIES; THE NUMBERS UNDER 295 ARE CORRELATIONS FOR THE 295 CITIES. THE CORRELATION IS IN EACH CASE OF THE MEASURE WHOSE IDENTIFICATION NUMBER (IN ITALICS) IS AT ITS LEFT WITH THE MEASURE WHOSE IDENTIFICATION NUMBER (IN ITALICS) IS ABOVE IT AT THE TOP OF THE SECTION OF THE TABLE WHERE THE CORRELATION IS

131		132		134		136		137		53		54		55		56	
200	295	200	295	200	295	200	295	200	295	200	295	200	295	200	295	200	295
131	64	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
132	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
133	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
134	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
135	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
136	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
137	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
138	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
139	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
140	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
141	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
142	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
143	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
144	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
145	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
146	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
147	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
148	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
149	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
150	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
151	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
152	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
153	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
154	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
155	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
156	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
157	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
158	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
159	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
160	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
161	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
162	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
163	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
164	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
165	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
166	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
167	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
168	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
169	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
170	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
171	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
172	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
173	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
174	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
175	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
176	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
177	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
178	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
179	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
180	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
181	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
182	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
183	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
184	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
185	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
186	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
187	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
188	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
189	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
190	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
191	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
192	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
193	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
194	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
195	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
196	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
197	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
198	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
199	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34
200	77	50	68	56	78	65	78	13	45	91	95	58	76	04	33	15	34

TABLE 5—Continued

	104		31		25		26		133		241		243		12		11	
	200	295	200	295	200	295	200	295	200	295	200	295	200	295	200	295	200	295
131	.25	.54																
132	.43	.48	.59	.61														
133	.15	.12	.38	.29	—	.59												
134	.16	.47	—	.14	.17	—	.20	—	.19	—	.21							
135	.17	.22	—	.14	.13	—	.01	—	—	.24	—	.10						
136	.18	.21	—	.19	—	—	.09	—	—	.21	—	.21						
137	.19	.23	—	.08	—	—	.31	—	—	.21	—	.37						
138	.20	.24	—	.27	.42	—	.31	—	—	.28	—	.23						
139	.21	.25	.27	.42	.29	.25	.31	.16	.09	.26	.54	.61						
140	.22	.26	.13	.29	.20	.16	.20	—	—	.06	—	.29						
141	.23	.27	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
142	.24	.28	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
143	.25	.29	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
144	.26	.30	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
145	.27	.31	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
146	.28	.32	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
147	.29	.33	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
148	.30	.34	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
149	.31	.35	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
150	.32	.36	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
151	.33	.37	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
152	.34	.38	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
153	.35	.39	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
154	.36	.40	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
155	.37	.41	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
156	.38	.42	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
157	.39	.43	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
158	.40	.44	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
159	.41	.45	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
160	.42	.46	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
161	.43	.47	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
162	.44	.48	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
163	.45	.49	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
164	.46	.50	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
165	.47	.51	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
166	.48	.52	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
167	.49	.53	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
168	.50	.54	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
169	.51	.55	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
170	.52	.56	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
171	.53	.57	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
172	.54	.58	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
173	.55	.59	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
174	.56	.60	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
175	.57	.61	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
176	.58	.62	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
177	.59	.63	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
178	.60	.64	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
179	.61	.65	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
180	.62	.66	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
181	.63	.67	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
182	.64	.68	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
183	.65	.69	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
184	.66	.70	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
185	.67	.71	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
186	.68	.72	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
187	.69	.73	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
188	.70	.74	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
189	.71	.75	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
190	.72	.76	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
191	.73	.77	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
192	.74	.78	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
193	.75	.79	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
194	.76	.80	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
195	.77	.81	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
196	.78	.82	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
197	.79	.83	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
198	.80	.84	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
199	.81	.85	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
200	.82	.86	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
201	.83	.87	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
202	.84	.88	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
203	.85	.89	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
204	.86	.90	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
205	.87	.91	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
206	.88	.92	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
207	.89	.93	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
208	.90	.94	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
209	.91	.95	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
210	.92	.96	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
211	.93	.97	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
212	.94	.98	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
213	.95	.99	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
214	.96	.100	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
215	.97	.101	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
216	.98	.102	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
217	.99	.103	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
218	.100	.104	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
219	.101	.105	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
220	.102	.106	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
221	.103	.107	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
222	.104	.108	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
223	.105	.109	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
224	.106	.110	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
225	.107	.111	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
226	.108	.112	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
227	.109	.113	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
228	.110	.114	.27	.29	.20	.20	.02	—	.06	.24	.38	.46						
229	.111	.115	.27	.29	.20	.20	.02											

TABLE 5A
INTERCORRELATIONS OF MEASURES OF WHICH G IS A COMPOSITE, IN STATES

	131	132	134	136	137	53a	54	55	56	21	22	24a	57	17	107	108	153	154
131																		
132	.61																	
134	.83	.30																
136	.81	.31	.43															
137	.66	.35	.70	.19														
53a	.61	.48	.60	.43	.67													
54	.75	.48	.65	.24	.34	.95												
55	.23	.03	.08	.31	.48	.51	.43											
56	.15	.18	.60	.66	.34	.64	.30	.57										
21	.79	.41	.43	.61	.34	.64	.71	.26	.07	.83								
22	.44	.19	.30	.61	.19	.73	.52	.46	.46	.21	-.07							
24a	.34	.34	.60	.03	.67	.66	.56	.43	.31	.34	.13	.58						
57	.50	.03	.60	.43	.67	.56	.51	.33	.20	.33	.26	.81	.26					
17	.45	.13	.43	.10	.48	.66								.50				
107	.63	.07	.86	.10	.90	.52	.34	.52	.63	.14	.19	.82	.52	.38	1.00	.71		
108	.63	.07	.86	.10	.90	.52	.34	.52	.63	.14	.19	.82	.52	.38	.71	.33	.57	
153	.50	.13	.75	.13	.66	.56	.44	.66	.44	.19	.08	.75	.66	.56	.33	.33		
154	.76	.57	.42	.76	.51	.57	.76	.39	.81	.75	.67	.13	.67	.48	.14	.14		
223	.71	.41	.48	.65	.35	.75	.88	.28	.14	.75	.65	.35	.48	.54	.56	.56	.35	.80
206	.44	.19	.48	.61	.34	.26	.38	.26	.13	.64	.50	.46	.26	.13	.39	.89	.71	.20
218	.38	.32	.75	.03	.78	.62	.38	.32	.44	.18	.10	.89	.44	.39	.89	.89	.44	.81
201	.66	.23	.65	.61	.61	.38	.59	.29	.42	.47	.47	.23	.59	.06	.53	.53		
98	.51	.33	.83	.17	.72	.51	.26	.33	.51	.26	.13	.87	.51	.45	.91	.91	.83	.26
99	.42	.10	.54	.03	.61	.38	.23	.10	.29	.06	.36	.82	.47	.57	.76	.76	.71	.90
102	.56	.24	.60	.61	.48	.66	.84	.56	.50	.71	.71	.87	.31	.80	.37	.37	.50	.84
103	.73	.13	.90	.50	.82	.82	.52	.34	.63	.40	.06	.73	.58	.32	.92	.92	.79	.57
104	.74	.00	.93	.10	.86	.86	.44	.44	.75	.19	.06	.73	.44	.38	.99	.99	.79	.45
31	.92	.52	.73	.82	.68	.58	.68	.27	.27	.78	.63	.27	.46	.34	.52	.52	.34	.96
26	.39	.13	.70	.30	.38	.72	.57	.87	.33	.51	.39	.46	.36	.67	.62	.62	.66	.51
25	.24	.24	.60	.10	.27	.80	.66	.80	.31	.27	.50	.76	.30	.67	.37	.37	.75	.26
153	.57	.45	.65	.71	.48	.83	.53	.61	.81	.65	.13	.13	.45	.39	.30	.39	.44	.56
217	.76	.13	.93	.43	.82	.62	.72	.67	.62	.62	.39	.57	.62	.20	.81	.81	.75	.62
218	.15	.15	.36	.03	.45	.45	.62	.15	.31	.34	.06	.97	.38	.71	.38	.38	.58	.20
12	.07	.20	.23	.43	.11	.67	.62	.33	.31	.24	.32	.27	.07	.13	.13	.13	.00	.50
11	.38	.26	.23	.31	.27	.66	.64	.44	.64	.64	.64	.97	.06	.67	.20	.20	.26	.51
16	.10	.10	.10	.03	.12	.13	.17	.50	.10	.03	.10	.39	.37	.06	.31	.31	.19	.26

TABLE 5A—Continued

	223	106	248	201	98	99	102	103	104	31	25	26	133	241	243	12	11	16
131																		
132																		
134																		
136																		
137																		
53a																		
54																		
55																		
56																		
21																		
22																		
24a																		
57																		
17																		
107																		
108																		
153																		
154																		
223																		
106	.48																	
248	.14	.44																
201	.48	.59	.42															
98	.07	.67	.93	.45														
99	.28	.29	.86	.42	.81													
102	.71	.66	.30	.71	.33	.03												
103	.41	.63	.82	.78	.87	.73	.68											
104	.28	.56	.86	.56	.91	.79	.50	.92										
31	.82	.78	.27	.85	.46	.13	.82	.63	.52	.46								
25	.64	.57	.77	.59	.67	.29	.56	.73	.66									
26	.41	.50	.77	.23	.67	.29	.43	.52	.44	.20	.91							
133	.28	.20	.26	.76	.39	.20	.62	.57	.51	.63	.33	.20						
211	.41	.67	.72	.72	.84	.51	.87	.68	.87	.68	.62	.62	.76					
213	—	.65	—	.15	—	.52	—	.45	—	.20	—	.59	.15	.33				
12	.41	.32	.38	—	.06	.03	.56	.27	.26	.20	.28	.45	.00	—	.38	.38		
19	.41	.44	.96	.42	.13	.10	.76	.20	.13	.52	.37	.56	.51	.23	.23	.31		
16	.00	.17	—	.17	—	.03	.03	.20	.06	.20	.10	.37	.39	.39	.00	.31	—	.03

TABLE 6

THE RELATIVE FREQUENCIES OF CORRELATION OF EACH DEGREE OF CLOSENESS
AMONG THE MEASURES CONSTITUTING THE G SCORE

Frequency, in percents			Frequency, in percents		
Correlation	200 cities	295 cities	Correlation	200 cities	295 cities
-.41 to -.45 . . .	0.3		.30 to .34 . . .	5.8	9.8
-.36 to -.40 . . .	0.15		.35 to .39 . . .	4.6	8.6
-.31 to -.35 . . .	1.35	.15	.40 to .44 . . .	5.9	4.8
-.26 to -.30 . . .	1.05		.45 to .49 . . .	3.2	6.0
-.21 to -.25 . . .	1.5	.75	.50 to .54 . . .	3.2	6.7
-.16 to -.20 . . .	3.6	1.13	.55 to .59 . . .	2.2	4.6
-.11 to -.15 . . .	3.3	1.3	.60 to .64 . . .	1.65	5.0
-.06 to -.10 . . .	4.4	2.9	.65 to .69 . . .	1.05	2.5
-.01 to -.05 . . .	5.9	3.3	.70 to .74 . . .	0.3	2.0
.00 to .04 . . .	5.3	3.2	.75 to .79 . . .	0.45	1.65
.05 to .09 . . .	10.5	4.6	.80 to .84 . . .		0.15
.10 to .14 . . .	9.0	6.3	.85 to .89 . . .		
.15 to .19 . . .	8.5	6.5	.90 to .94 . . .	0.3	
.20 to .24 . . .	8.0	8.8	.95 to .9915	0.15
.25 to .29 . . .	8.6	9.1	1.00 to 1.04 . . .		0.15

The closeness of the affiliation of each of the items or traits with G, I and P is shown by the correlations presented in TABLE 7. Two groups of cities are used, numbering 295 and 200. The former includes all cities with populations of over 30,000 and under 500,000 in 1930, except that, for special reasons, I omitted the resort cities Atlantic City, Miami, and St. Petersburg, and replaced Washington by Milwaukee, and counted Covington twice.* The latter excludes 55 cities which adjoin larger cities and the 40 cities of Virginia, North Carolina, South Carolina, Georgia, Florida, Kentucky, Tennessee, Alabama, Mississippi, and Louisiana.

Other correlations of importance are presented in TABLE 8.

* Covington was counted twice in the 295 cities because it occurred once among the 40 cities of the Old South and once among the 55 cities adjoining larger cities. Both of these groups were investigated in various ways and much labor was saved by combining their data unchanged in the 295 cities. There being only one such case, it seemed inadvisable to treat the entry with rigorous accuracy. The only harm done is that Covington has twice as much weight as other cities have in the correlations involving 295 cities.

TABLE 7

THE CORRELATIONS OF EACH ITEM WITH G, I, AND P. THE TWO GROUPS OF CITIES AND THE STATES ARE INDICATED BY 200, 295, AND S RESPECTIVELY

Identification Number	Item Description	With G (general goodness)		With I (per capita private income)		With P (personal qualities)	
		200	295 S	200	295 S	200	295 S
1	Population in 1930		.16 .06		.37 .25		-.28 -.17
2	Population in 1900		.08 -.03		.13 .03		-.16 -.06
3	Growth: population in 1930 ÷ by population in 1900		.02 .11		.20 .15		.36
4	Latitude N		.57 .67		.13 .35		.50
5	Longitude W						-.06
6	Taxable wealth		.28 .33		.51 .48		-.13 .02
7	Taxable wealth in real estate only		.27 .43		.59 .61		-.18 .10
8	Interest rate on public debt						
9	Density of population (persons per acre)		-.05 .08		.48 .48		-.35 -.13
10	Score for natural advantages		.08 -.18		.09 -.18		-.02 -.18
11	Value of public property minus public debt		.44 .47		-.03 .15		.38
12	Value of public property in schools, parks, etc.		.76 .74		.44 .57		.32
13	Public debt—gross municipal		-.03 .02		-.44 -.30		.00
14	Public debt—gross public service		.11 -.06		.19 -.02		-.17 -.31
15	Public debt—net		.23 .09		.61 .36		-.32 -.40
16	Value of public property in schools, etc., divided by value of public property in jails, etc.		.28 .41		.15 -.26		.25 .36
17	Average of parks		.32 .24		.23 .14		.83
21	Percentage of persons 16-17 attending school		.59 .71		.11 .34		.17
22	Percentage of persons 18-20 attending school		.49 .55		.07 .26		.13
23	Average salary of high-school teachers		.45 .57		.80 .76		-.11 .21
24	Average salary of elementary-school teachers		.40 .55		.52 .63		-.09 .17
24a	Average salary of all teachers and supervisors						
25	Circulation of three magazines		.58 .55		.57 .47		.71
26	Circulation of the Literary Digest		.33 .26		.13 .15		.86
29	Circulation of McFadden publications		.20 .22		.31 .22		.23
30	High-school graduates in 1934		.32 .43		-.16 .09		.39
31	Percentage of illiterates all ages > 10 (reversed)		.37 .52		-.21 .20		.26
							.75
							.93

TABLE 7—Continued

Identifi- cation Number	Item Description	With G (general goodness)		With I (per capita private income)		With P (personal qualities)	
		200	295	200	295	200	295
32	Percentage of illiterates 10-14 (reversed).....	.29	.60	.16	.37	.26	.56
33	Percentage of illiterates 15-24 (reversed).....	.53	.65	-.21	.19	.78	.81
41	Public library: circulation.....	.61	.08	.13	.85	.57	.73
43	Public expenditures for highways and light.....	.23	.33	.16	.21	-.08	.16
45	Public expenditures for sanitation.....	.16	.12	.50	.43	-.35	.31
46	Public expenditures for general government.....	.25	.35	.55	.55	-.19	-.03
47	Public expenditures for police.....	.33	.39	.63	.55	-.30	-.14
48	Public expenditures for fire.....	.35	.45	.22	.32	.05	.16
49	Public expenditures for health.....	.34	.33	.57	.44	-.20	.15
50	Public expenditures for public safety.....	.14	.30	.45	.48	-.21	-.06
51	Public expenditures for items 43 to 50.....	.38	.45	.56	.51	-.26	-.06
53	Public expenditures for schools.....	.54	.73	.55	.70	.13	.38
54	Public expenditures for teachers' salaries.....	.51	.70	.57	.69	.13	.34
55	Public expenditures for text-books and supplies.....	.32	.54	.30	.47	.27	.47
56	Public expenditures for libraries.....	.54	.66	.28	.47	.29	.51
57	Public expenditures for recreation.....	.52	.55	.66	.60	.26	.30
59	Wholesale sales, 1933.....	.09	-.28	.17	-.21	.00	-.28
60	Retail sales, 1933.....	.26	.16	.43	.27	.14	.18
61	Retail sales, 1930.....	.38	.34	.56	.44	.29	.40
62	Retail sales, food stores.....	.25	.44	.52	.65	.13	.41
64	Retail sales, cigar stores.....	.40	.36	.35	.29	.43	.41
65	Retail sales, drug stores.....	.16	-.05	.31	.08	.26	.08
67	Retail sales, auto and accessories.....	.34	.21	.26	.25	.43	.41
68	Retail sales, radio and music stores.....	.00	.02	-.02	.04	.06	.25
69	Retail sales, book stores.....	.29	.21	.34	.29	.14	.25
70	Retail sales, florists.....	.28	.06	.43	.18	.11	.11
71	Retail sales, jewelry stores.....	-.09	-.27	.17	-.05	-.06	-.16
74	Retail sales, second-hand stores.....	.20	.10	.34	.18	.02	.06
75	Services amusement and hotel receipts, 1933.....	.42	.31	.41	.28	.38	.31
76	Postal receipts in 1934.....						

TABLE 7—Continued

Identifi- cation Number	Item Description	With G (general goodness)		With I (per capita private income)		With P (personal qualities)	
		200	295	S	200	295	S
77	Percentage which sales of food stores were of the sales of all retail stores.....	-.03	.27	.13	-.17	.15	.67
78	Percentage which sales of auto stores were of the sales of all retail stores.....	.02	.08		-.22	-.09	
79	Percentage which sales of radio and music stores were of the sales of all retail stores.....	.00	.01	.53	-.05	.02	.75
81	Percentage which sales of cigar stores were of the sales of all retail stores.....	.32	.24	.92	.22	.14	.71
82	Percentage which sales of drug stores were of the sales of all retail stores.....	-.28	-.35	-.73	-.21	-.32	-.58
83	Percentage which sales of florists were of the sales of all retail stores.....	.18	.17		.16	.10	
84	Percentage which sales of jewelry stores were of the sales of all retail stores.....	.08	-.22		.34	.02	
87	Percentage which sales of second-hand stores were of the sales of all retail stores.....	-.17	-.31		.06	-.14	
88	Percentage which sales of "single" stores were of all retail sales.....	.06	-.17	.13	-.11	-.26	.26
89	Percentage which sales of "chain" stores were of all retail sales.....	-.03	.15	.20	.14	.18	.57
91	Number of cigar stores.....	.14	.25		.00	.19	
92	Number of drug stores.....	.23	.01		.13	-.05	.20
93	Number of jewelry stores.....	.17	.14		.28	.25	.08
97	Domestic installations of electricity by Source A.....	.54	.65	.50	.14	.43	.79
98	Domestic installations of electricity by Source B.....	.51	.57		.23	.42	.59
99	Domestic installations of gas.....	.45	.62	.32	.57	.73	.75
100	Ford cars.....	.07	.10	.50	-.23	-.16	.13
101	Cars other than Ford.....	.47	.58	.72	.40	.52	.51
102	Item 100 plus item 101.....	.34	.47	.75	.48	.34	.32
103	Telephones.....	.71	.66	.63	.13	.35	.63
104	Radios.....	.73	.87	.75	.40	.70	.66
105	Relief: percentage of funds contributed by the federal government.....			-.71			-.61
106	Homes owned.....	.51	.55	.50	-.11	.16	.50
							-.79
							.86
							.71

TABLE 7—Continued

Identifi- cation Number	Item Description	With G (general goodness)		With I (per capita private income)		With P (personal qualities)	
		200	295	S	200	295	S
107	Rarity of extreme poverty—5 percentile rental.	.54	.73	.66	.74	.81	.75
107a	Rarity of extreme poverty—percentage of rentals under \$10 a month.	.40	.66		.70	.80	
108	Rarity of less extreme poverty—10 percentile rental.	.56	.74	.66	.72	.81	.75
109	50 percentile rental.	.48	.73		.70	.84	
110	75 percentile rental.	.45	.63		.82	.83	
111	90 percentile rental.	.31	.51		.80	.83	
112	95 percentile rental.	.19	.42	.13	.74	.77	.50
113	Average rental.	.44	.66	.57	.83	.87	.76
114	Variability in income—95 percentile rental minus 5 percentile rental.	.16	.31		.76	.69	
115	Variability in income—95 percentile ÷ 5 percentile rental.	— .45	— .55		— .02	— .25	
115a	Average of scores in 114 and 115.	— .26	— .28				
116a	Average of scores in 116a and 120d.	— .24	— .24		.45	.23	
116b	90 percentile rental—10 percentile rental.	.24	.37				
117	90 percentile rental ÷ 10 percentile rental.	— .48	— .55		— .52	— .51	
120a	95 percentile rental—50 percentile rental.	.17	.26		— .33	— .05	
120b	50 percentile rental—5 percentile rental.	.40	.63		— .61	— .65	
120d	Average of scores in 116 and 117.	— .28	— .32				
124	Number of church members aged 13 or over, all.	— .20	— .22	— .13	.00	— .03	.20
125	Number of church members aged 13 or over, Jewish congregations.	— .04	.01	.26	.44	.32	.71
126	Number of church members aged 13 or over, Roman Catholic.	.05	.25	.80	.28	.41	.70
127	Number of church members aged 13 or over, Unitarian, Universalist and Christian Science.	.45	.57	.61	.14	.36	.61
128	Males per 100 females.	.14	.16	.61	.25	.23	.13
129	Births per 1000 population.	— .37	— .43	— .57	— .03	— .21	— .77
130	Births per 1000 females aged 20-44.	— .39	— .34	.53	— .17	— .18	— .69
131	Infant death rate (reversed).	.75	.86	.89	.32	.56	.32
132	General death rate (reversed).	.40	.56	.55	.23	.43	.19
133	Deaths from syphilis (reversed).	.22	.48	.50	— .17	.24	.13
134	Deaths from typhoid (reversed).	.52	.67	.79	.20	.45	.61
							.08
							.37
							.86

TABLE 7—Continued

Identifi- cation Number	Item Description	With G (general goodness)		With I (per capita private income)		With P (personal qualities)	
		200	295 S	200	295 S	200	295 S
135	Deaths from diabetes (reversed).....	.06	.03 — .61	-.02	-.01 — .71	-.19	-.26 — .61
136	Deaths from appendicitis (reversed).....	.20	.37 — .51	.16	.25 — .62	-.20	.08 — .72
137	Deaths from puerperal diseases (reversed).....	.45	.64 .79	.14	.44 .61	-.03	.32 .71
138	Deaths from certain venereal diseases other than syphilis (re- versed).....	.19	.39	.17	.37	-.29	.08
140	Median size of family in 1980.....	-.29	-.07 — .61	-.07	.06 — .61	-.51	-.29 — .61
141	Average size of family in 1900, 1910, and 1930.....	-.38	-.21 — .50	-.02	-.05 — .50	-.60	-.35 — .50
142	Increase in average size of family 1900 to 1920.....	.02	-.07 — .28	.31	.05 .00	-.29	-.32 — .07
143	Percentage of families native-born white.....	.16	.22 — .19	-.11	.03 — .19	.43	.49 .82
144	Percentage of families foreign-born white.....	.26	.51 .78	.32	.50 .82	-.16	.21 .82
145	Percentage of families Negro.....	-.46	-.60 — .61	.02	-.25 — .50	-.44	-.60 — .79
146	Percentage of the population under 15.....	-.45	-.12	-.29	-.41	-.48	-.49
147	Percentage of the population under 10.....	-.44	-.41 — .62	-.43	-.47 — .84	-.41	-.44 — .51
147a	Percentage of the population under 10, Male.....	-.61	-.53 — .75	-.62	-.56 — .90	-.35	-.46 — .54
147b	Percentage of the population under 10, Female.....	-.43	-.43 — .50	-.36	-.44 — .79	-.47	-.49 — .50
148	Percentage of the population 65 or over.....	.28	.33 .48	-.08	.12 .60	.57	.61 .60
148a	Percentage of the population 65 or over, Male.....	.31	.37 .65	-.20	.05 .43	.61	.66 .75
148b	Percentage of the population 65 or over, Female.....	.24	.40 .44	-.02	.27 .66	.49	.64 .66
149	Percentage of the population 20-30, Male.....	.16	-.05 .38	.34	.21 .79	.11	-.33 — .13
149a	Percentage of the population 20-30, Male.....	.40	.41 .71	.30	.43 .71	.37	.57 .61
149b	Percentage of the population 20-30, Female.....	.11	-.07 .26	.31	.09 .79	-.08	-.24 .00
150	Percentage of the population 40-64, Male.....	.31	.46 .50	.14	.44 .79	.40	.64 .50
150a	Percentage of the population 40-64, Male.....	-.32	-.32 — .50	-.09	-.23 — .71	-.38	-.40 — .38
150b	Percentage of the population 40-64, Female.....	-.34	-.33 — .61	-.08	-.36 — .79	-.40	-.58 — .50
150c	Percentage of the population 15-19.....	.20	.02	.25	.26	-.02	-.26
151	Males aged 10 or over gainfully employed.....	.08	.14	.34	-.03	-.17	-.38
152	Females aged 10 or over gainfully employed.....	.45	.62 .66	.11	.35 .06	.34	.51 .55
153	Males aged 10-14 gainfully employed (reversed).....	.54	.63 .79	.02	-.27 .13	.59	.79 .86
154	Females aged 10-14 gainfully employed (reversed).....	.05	-.25 — .26	.06	-.22 .38	.13	-.14 — .38
155	Married women gainfully employed.....	.00	-.01 .10	.05	.02 .60	-.16	-.01 .10
156	Factory workers.....	.00	-.01 .10	.05	.02 .60	-.16	-.01 .10

TABLE 7—Continued

Identifi- cation Number	Item Description	With G (general goodness)		With I (per capita private income)		With P (personal qualities)	
		200	295	200	295	200	295
157	Unemployed in 1980; no job.		.26				
158	Unemployed in 1980; with jobs, but laid off.	-.24	-.18				
162	Clergy, Male.	-.19	-.47	-.23	-.48	.22	-.15
163	Clergy, Female.	.11	-.05	-.03	-.17	.26	.10
164	Physicians, Male.	.10	-.01	.21	.44	.25	.10
165	Physicians, Female.	.10	.27	.23	.32	.13	.36
166	Nurses, Male.	.00	.10	.17	.22	-.03	-.01
167	Nurses, Female.	.32	.25	.16	.13	.32	.29
168	Domestic servants, Male.	.08	-.17	.34	.04	-.12	-.32
169	Domestic servants, Female.	-.06	-.25	.19	.04	-.02	-.22
171	Teachers, Male.	.14	.17	.00	.28	.46	.55
172	Teachers, Female.	.38	.23	-.02	.09	.05	.18
173	Musicians, Male.	.35	.40	.00	.13	.60	.53
174	Musicians, Female.	.28	.44	.17	.40	.00	.25
177	Dentists, Male.	.45	.64	.14	.39	.66	.75
178	Dentists, Female.	.08	.13	.29	.28	-.16	.04
179	Lawyers, Male.	.06	.01	.29	.10	.00	-.08
180	Lawyers, Female.	.09	.17	.29	.36	-.09	-.08
181	Actors, Male.	-.03	.01	-.11	-.01	.13	.12
182	Actors, Female.	.23	.35	.14	.33	.14	.21
183	Artists, sculptors and teachers of art, Male.	.40	.48	.44	.52	.22	.29
184	Artists, sculptors and teachers of art, Female.	.28	.39	.26	.40	.28	.30
185	Public service employees, Male.	.28	.16	.37	.21	-.16	-.28
186	Public service employees, Female.	.07	.03	-.04	-.04	.17	.09
187	All professional except teachers, Male.	.29	.43	.51	.55	.20	.34
188	All professional except teachers, Female.	.22	.25	.17	.21	.28	.29
189	Police, Male.	.13	.31	.44	.43	-.48	-.22
190	Architects, Male.	.28	.38	.43	.46	-.02	.12
191	Designers, Male.	.31	.54	.60	.67	.00	.33
192	Osteopaths, Male.	.25	.39	-.02	.26	.51	.63
193	Technical Engineers, Male.	.30	.51	.43	.55	.03	.32
194	Veterinary Physicians, Male.	.07	-.02	-.37	-.30	.53	.31

TABLE 7—Continued

Identifi- cation Number	Item Description	With G (general goodness)		With I (per capita private income)		With P (personal qualities)	
		200	295 S	200	295 S	200	295 S
195	Police, Female.....						
196	Architects, Female.....						
197	Designers, Female.....	.44	.57 .50	.28	.47 .86	.26	.35 .50
198	Osteopaths, Female.....	.14	.20 .36	.06	.20 .43	.29	.30 .60
199	Technical Engineers, Female.....						
200	Veterinaries, Female.....						
201	Excess of physicians, nurses and teachers over male domestic servants.....	.29	.34 .59	-.13	.05 .23	.49	.54 .94
202	Number of female clergy divided by the number of male clergy	.20	.17 .48	-.06	.03 .43	.29	.25 .60
203	Sum of items 195, 196, 199 and 200.....	.19	.23 .38	.20	.25 .86	.06	.10 .50
204	178 ÷ 177; Ratio of female dentists to male.....	.06	.12 .10	.31	.29 .65	-.17	.03 -.03
205	180 ÷ 179; Ratio of female lawyers to male.....	.02	.18 .34	.25	.26 .58	-.17	-.06 .27
206	118 ÷ 117; Ratio of female teachers to male.....	-.17	-.44 .13	-.03	-.29 .00	-.38	-.55 .26
207	182 ÷ 181; Ratio of female actors to male.....	.14	.35 .50	.31	.41 .61	-.02	.14 .26
207a	184 ÷ 183; Ratio of female artists, etc. to male.....	-.02	-.09 -.20	-.16	.22 .52	.02	-.03 .20
207b	186 ÷ 185; Ratio of female public service employees to male..	.11	.04 .38	-.22	-.17 -.13	.38	.29 .38
207c	188 ÷ 187; Ratio of female professional workers except teachers to male.....	.11	-.03 .48	-.19	-.29 .17	.35	.17 .79
207d	177 ÷ 179.....	.23	.40 .55	-.13	.13 .44	.40	.60 .89
208	Percentage of population in Who's Who, 1930.....	.16	.02 .23	.22	.06 .86	.08	.01 .30
209	Percentage of population in Who's Who, 1910.....	.22	.10 .38	.35	.21 .79	.03	.10 .38
210	Percentage of population in Who's Who, Gain 1910 to 1930....	-.02	.07 -.19	.08	.01 -.32	.03	.05 -.32
211	Percentage of population in American Association for the Advancement of Science.....	.17	.18 .45	.28	.27 .93	.02	.10 .33
220	Average salary, full-time workers in retail stores all.....	.56	.72 .74	.78	.85 .94	.00	.23 .42
221	Average salary, full-time workers retail stores national chain stores.....	.49	.44	.55	.55	.06	.14
222	Sum of items 220 and 221.....	.55	.63	.75	.78	.08	.23
223	Average wage in manufacturing plants (Av. of 1929 and 1931).....	.48	.62 .75	.49	.65 .41	.19	.36 .54
229	Income (by Curtis Co. estimates).....	.48	.63	.90	.90	-.02	.32
230	Income (Crowell Co. estimates of total Income).....	.28	.25	.57	.51	.03	.12

TABLE 7—Continued

Identifi- cation Number	Item Description	With G (general goodness)		With I (per capita private income)		With P (personal qualities)	
		200	295	200	295	200	295
231	Income (Crowell Co. estimates of marginal income).....					-.23	.04
232	Income (Crowell Co. estimates of effective income).....	.28	.42	.77	.70	.03	.18
233	Income tax returns (average of 1930 and 1931).....	.29	.25	.43	.39	.00	.27
234	Income tax returns (average of 1930 and 1931).....	.48	.59	.80	.81	.00	.27
235	Income tax returns for \$5000 or over (estimated from reports for counties).....		.38		.92		.38
237	Value added to products by manufacture.....	.16	.18	.29	.19	-.25	.04
238	Bank deposits (Curtis Co. estimates).....	.07	.21	.38	.38	-.30	-.02
241	Deaths from homicide (reversed).....	.41	.63	.03	.35	.22	.47
242	Deaths from suicide (reversed).....	-.08	-.04	.20	-.10	-.35	-.15
243	Deaths from automobile accidents (reversed).....	.26	.37	.00	.17	-.11	.10
244	Illegitimate births (reversed).....	.25	.51	-.02	.38	.09	.38
244a	Illegitimate births (reversed) omitting cities where reports are not mandatory.....					.02	.31
248	Support of the Y. M. C. A.....	.17	.46	-.11	.32	.00	.19
249	Boy Scouts.....	.16	.11	.29	.29	.00	.19
250	Outside benevolences of churches divided by their expenditures for their own buildings, ministers' chairs, etc.....	.53	.58	.57	.59	.26	.45
251	Kiwanis Club members.....	.13	.07	.26	.12	.06	.07
252	Rotary Club members.....	-.02	-.04	-.19	-.17	.35	.32
255	Percentage of the total public expenditures spent for general government.....	-.01	-.13	-.27	-.32	.35	.24
256	Percentage of total public expenditures spent for police.....	.09	.24	.02	-.09	-.03	-.20
257	Percentage of total public expenditures spent for health.....	.29	.29	.25	.03	-.46	.56
258	Percentage of total public expenditures spent for sanitation.....	.05	-.06	.37	.15	.17	.26
259	Percentage of total public expenditures spent for charities.....	-.16	-.19	.38	.24	-.43	.35
260	Percentage of total public expenditures spent for schools.....	.03	.08	.20	-.02	-.34	.33
261	Percentage of total public expenditures spent for libraries.....	.05	.22	-.25	-.01	.35	.47
262	Percentage of total public expenditures spent for recreation.....	.44	.54	.06	.24	.46	.57
263	Employment of a city manager.....	.37	.31	.17	.14	.31	.24
264	Expenditures for civil service.....						
265	Expenditures for city planning.....						

TABLE 7—Continued

Identifi- cation Number	Item Description	With G (general goodness)		With I (per capita private income)		With P (personal qualities)	
		200	295	200	295	200	295
266	Expenditures for vital statistics.						
267	Expenditures for public convenience stations.						
268	Expenditures for a probation system.						
269	Expenditures for mother's aid.33	.31	-.23	-.02
270	Composite of items 263 to 269 inclusive.28	.28	.32	.36	-.19	.00
271	Composite of items 264 to 268 inclusive.28	.24				
401	Ratio of salaries of teachers to salaries of employees in retail stores: sum of items 23 and 24 divided by item 220.16	.35	.43	.49	-.16	.13
402	Ratio of salaries of teachers to salaries in national chain stores; sum of items 23 and 24 divided by item 221.18	.39	.33	.44		
403	Superiority of salaries of teachers to salaries of policemen and firemen: deviations from median of item 23 plus 24, 24 minus the deviations from the median of item 223 and 225.21	.21	.47	.42	.08	.21
404	Weighted average of policemen's and firemen's salaries.40	.62	.52	.66	-.20	.15
405	Realtive excess of florists' sales over drug sales.09	.31	.00	.24	-.11	.18
406	Relative excess of payments for rent over service, amusement and hotel receipts.28	.51	.44	.60	-.09	.24
407	Relative excess of item 153 over item 152.16	.33	.11	.36	.00	.36
408	Relative excess of artists, designers, and engineers over clergy- men and lawyers.26	.51	.44	.60	-.09	.24
411	Frequency of eminent men and men of science: composite of items 208, 209 and 211.26	.18	.32	.23	.12	.11
412	Composite of homicide rate (reversed), typhoid death rate (re- versed) and score for items 264 to 268.51	.69	.38	.57	.03	.39
413	Composite of items 11 and 12 (value of public property minus public debt and value of schools, libraries, parks, etc.)66	.72	.29	.46	.46	.66
414	Composite of items 244 and 138 (illegitimate births reversed and death-rate from non-syphilitic venereal diseases)37	.60	.12	.44	-.07	.32
415	Composite of items 131 and 132 (infant death rate reversed and general death rate reversed)56	.71	.28	.50	.28	.43

It will be well for the reader to remember that in two affiliated items or traits the percentage of the variation in one which is attributable to the variation in the other equals r^2 and so is very low for small coefficients, being:

.01	when the correlation is .10
.0225	when the correlation is .15
.04	when the correlation is .20
.0625	when the correlation is .25
.09	when the correlation is .30
.1225	when the correlation is .35
.16	when the correlation is .40
.25	when the correlation is .50
.36	when the correlation is .60

These correlations are in one sense entirely reliable as measures of the conditions that existed in the specified cities; there is no sampling error since they include them all. But in another sense we may estimate their unreliabilities, as follows: For 295 cities the P.E.'s for $r = .10, .20, .30, \dots, .90$ are respectively: $-.122, .119, .114, .107, .098, .086, .072, .055$ and $.033$. For 200 cities the corresponding P.E.'s are: $.147, .143, .137, .129, .118, .104, .087, .066$ and $.040$. For 48 states they are: $.30, .29, .28, .26, .24, .21, .175, .134$, and $.080$. These estimates are for the probable divergences of what we did find from what would be found in an infinite number of enquiries such as ours if such could have been made in other countries strictly comparable to the United States. The largeness of these P.E.'s is due to the use of the cosine πU formula.

TABLE 8

THE FIRST OF THE CORRELATIONS IS THAT FOR THE 200 CITIES, THE SECOND IS THAT FOR THE 295 CITIES

(*w.* = with; *rev.* = reversed)

Item

1	<i>w.</i> 146; $-.09, -.15$. <i>w.</i> 145 <i>rev.</i> ; $-.05, -.13$. <i>w.</i> 411; .32, .26
4	<i>w.</i> 146; .17, .12. <i>w.</i> 145 <i>rev.</i> ; .73, .80. <i>w.</i> 124; .18, .06
5	<i>w.</i> 146; $-.46, -.43$. <i>w.</i> 145 <i>rev.</i> ; $-.08, -.16$. <i>w.</i> 130; $-.42, -.14$
6	<i>w.</i> 145 <i>rev.</i> ; $-.05, .00$
7	<i>w.</i> 145 <i>rev.</i> ; $-.02, -.06$
9	<i>w.</i> 146; $-.14, -.11$
10	<i>w.</i> 1; .05, .20. <i>w.</i> 4; .25, $-.10$. <i>w.</i> 6; $-.02, -.08$
11	<i>w.</i> 145 <i>rev.</i> ; .46, .48
12	<i>w.</i> 145 <i>rev.</i> ; .26, .37. <i>w.</i> 1; .34, .27. <i>w.</i> 11; .43, .53. <i>w.</i> 124; .14, .08
21	<i>w.</i> 146; $-.31, -.41$. <i>w.</i> 124; $-.41, -.37$
22	<i>w.</i> 146; $-.54, -.51$. <i>w.</i> 124; $-.41, -.24$
23	<i>w.</i> 146; $-.18, -.28$. <i>w.</i> 1; .44, .37

TABLE 8—(continued)

Item	
24	<i>w. 146</i> ; -.09, -.18. <i>w. 1</i> ; .51, .38
25	<i>w. 124</i> ; -.30, -.17
30	<i>w. 31</i> ; in 295 cities, .37. <i>w. 33</i> ; in 295 cities, .38. <i>w. 41</i> ; in 295 cities, .41
31	<i>w. 146</i> ; -.54, -.51. <i>w. 124</i> ; -.62, -.63. <i>w. 145 rev.</i> ; -.11, -.20. <i>w. 33</i> ; in 295 cities, .83
32	<i>w. 146</i> ; -.08, -.11
33	<i>w. 146</i> ; -.47, -.41. <i>w. 124</i> ; -.35, -.36. <i>w. 145 rev.</i> ; .40, .60, in 182 cities, .29
41	<i>w. 124</i> ; -.36, -.26
55	<i>w. 1</i> ; -.03, -.14
59	<i>w. 146</i> ; -.43, -.20
60	<i>w. 146</i> ; -.57, -.49
62	<i>w. 1</i> ; -.16, .04
64	<i>w. 146</i> ; -.31, -.35. <i>w. 1</i> ; .06, .12
65	<i>w. 146</i> ; -.39, -.36. <i>w. 1</i> ; .02, .02
67	<i>w. 146</i> ; -.45, -.51. <i>w. 1</i> ; -.26, -.20
68	<i>w. 146</i> ; -.06, -.13. <i>w. 1</i> ; -.51, -.42
70	<i>w. 146</i> ; -.23, -.26
71	<i>w. 146</i> ; -.32, -.27. <i>w. 1</i> ; .09, .06
75	<i>w. 145 rev.</i> ; -.13, -.19
76	<i>w. 145 rev.</i> ; -.06, -.10. <i>w. 1</i> ; .29, .23
78	<i>w. 146</i> ; -.14, -.15
79	<i>w. 146</i> ; .06, -.03
81	<i>w. 146</i> ; -.23, -.16
82	<i>w. 146</i> ; .03, -.02
83	<i>w. 146</i> ; .03, -.11
84	<i>w. 146</i> ; -.23, -.11
103	<i>w. 106</i> ; in 295 cities, .60
106	<i>w. 146</i> ; -.45, -.44. <i>w. 4</i> ; .31, .33. <i>w. 103</i> ; in 295 cities, .60. <i>w. 124</i> ; -.29, -.27
107	<i>w. 146</i> ; -.06, -.14. <i>w. 109</i> ; .92, .96. <i>w. 107a</i> ; .97, .98
109	<i>w. 146</i> ; -.09, -.17. <i>w. 145 rev.</i> ; .26, .40. <i>w. 1</i> ; .31, .18. <i>w. 107a</i> ; .87, .94. <i>w. 114</i> ; .72, .69. <i>w. 115</i> ; -.22, -.47
113	<i>w. 1</i> ; .29, .16
114	<i>w. 146</i> ; .03, -.08. <i>w. 107</i> ; .64, .63
115	<i>w. 146</i> ; .16, .14. <i>w. 107</i> ; -.48, -.59
115a	<i>w. 120d</i> ; .94, .93
115b	<i>w. 146</i> ; .14, .08. <i>w. 145 rev.</i> ; -.03, -.27. <i>w. 107</i> ; .14, .03. <i>w. 109</i> ; .31, .08
116	<i>w. 107</i> ; .70, .67. <i>w. 109</i> ; .76, .78
117	<i>w. 107</i> ; -.64, -.56. <i>w. 109</i> ; -.34, -.45
120a	<i>w. 107</i> ; .66, .63. <i>w. 109</i> ; .68, .63
120b	<i>w. 107</i> ; .76, .81. <i>w. 109</i> ; .93, .94
124	<i>w. 146</i> ; .36, .28. <i>w. 145 rev.</i> ; .10, -.06. <i>w. 21</i> ; -.41, -.37. <i>w. 22</i> ; -.41, -.24. <i>w. 25</i> ; -.30, -.17. <i>w. 31</i> ; -.63, -.62. <i>w. 33</i> ; -.35, -.36. <i>w. 106</i> ; -.30, -.27. <i>w. 144</i> ; .50, .25. <i>w. 143</i> ; -.54, -.33. <i>w. 4</i> ; .16, .06. <i>w. 130</i> ; .36, .27. <i>w. 131</i> ; -.03, -.05. <i>w. 133 rev.</i> ; .31, .17. <i>w. 138 rev.</i> ; .44, .28. <i>w. 153 rev.</i> ; .08, -.08. <i>w. 154 rev.</i> ; -.44, -.48. <i>w. 156</i> ; .24, .24. <i>w. 244 rev.</i> ; .19, .06
125	<i>w. 146</i> ; .26, .16
126	<i>w. 146</i> ; .13, .05
127	<i>w. 146</i> ; -.48, -.43
128	<i>w. 145 rev.</i> ; -.06, .18
129	<i>w. 146</i> ; .43, .36
130	<i>w. 146</i> ; .43, .39. <i>w. 145 rev.</i> ; -.03, -.04. <i>w. 9</i> ; -.12, -.06. <i>w. 124</i> ; .36, .27. <i>w. 131</i> ; -.22, -.22. <i>w. 133</i> ; .00, -.07. <i>w. 140</i> ; .46, .26. <i>w. 141</i> ; .05, .01. <i>w. 142</i> ; .32, .18. <i>w. 143</i> ; -.22, -.02. <i>w. 144</i> ; .22, .09. <i>w. 156</i> ; .17, .27. <i>w. 153 rev.</i> ; -.03, -.02. <i>w. 154 rev.</i> ; -.31, -.27. <i>w. 244</i> ; -.07, -.13
131	<i>w. 146</i> ; -.22, -.25. <i>w. 145 rev.</i> ; .63, .72. <i>w. 1</i> ; .03, .08. <i>w. 124</i> ; -.02, -.05. <i>w. 132</i> ; .48, .64. <i>w. 130</i> ; -.22, -.22. <i>w. 144</i> ; .52, .67. <i>w. 244</i> ; .48, .65. <i>w. 412</i> ; .51, .67. <i>w. 413</i> ; .37, .53
132	<i>w. 146</i> ; .19, .07. <i>w. 143</i> ; .31, .58. <i>w. 244</i> ; .06, .24. <i>w. 412</i> ; .37, .51

TABLE 8—(continued)

Item	
133	<i>w. 146</i> ; .16, — .03. <i>w. 124</i> ; .31, .17. <i>w. 166</i> ; in 295 cities, .62.
134	<i>w. 145 rev.</i> ; .50, .69. <i>w. 1</i> ; .20, .16. <i>w. 270</i> ; .41, .31
136	<i>w. 144</i> ; .38, .58
137	<i>w. 145 rev.</i> ; .41, .58. <i>w. 1</i> ; .13, .00. <i>w. 144</i> ; .34, .63. <i>w. 270</i> ; .47, .42
138	<i>w. 124</i> ; .44, .28
140	<i>w. 146</i> ; .90, .78. <i>w. 130</i> ; .41, .14
141	<i>w. 145 rev.</i> ; .02, — .03. <i>w. 4</i> ; — .20, — .10. <i>w. 130</i> ; .05, .01
142	<i>w. 130</i> ; .32, .18
143	<i>w. 146</i> ; — .46, — .48. <i>w. 145 rev.</i> ; — .27, .00. <i>w. 124</i> ; — .54, — .33. <i>w. 130</i> ; — .13, .01.
144	<i>w. 146</i> ; .34, .23. <i>w. 145 rev.</i> ; .46, .58. <i>w. 124</i> ; .50, .25. <i>w. 130</i> ; .15, — .01. <i>w. 131</i> ; .52, .67. <i>w. 132</i> ; .31, .58. <i>w. 137</i> ; .34, .63. <i>w. 156</i> ; .48, .44. <i>w. 171</i> ; .38, .58. <i>w. 244</i> ; .40, .60. <i>w. 412</i> ; .51, .66. <i>w. 413</i> ; .22, .37. <i>w. 414</i> ; .53, .68
145	<i>w. 146</i> ; .05, .01; also see the other item in the correlation.
146	See the other item in the correlation
147	<i>w. 145 rev.</i> ; — .16, — .16. <i>w. 1</i> ; — .08, .12. <i>w. 4</i> ; .00, .03. <i>w. 130</i> ; .38, .32. <i>w. 140</i> ; .78, .71. <i>w. 141</i> ; .62, .54. <i>w. 412</i> ; — .32, — .29.
147a	<i>w. 145 rev.</i> ; — .17, — .24
147b	<i>w. 145 rev.</i> ; — .19, — .18
148	<i>w. 145 rev.</i> ; .40, .43. <i>w. 4</i> ; .16, .23. <i>w. 124</i> ; — .03, .02. <i>w. 130</i> ; — .08, .05. <i>w. 140</i> ; — .58, — .48. <i>w. 141</i> ; — .64, — .47. <i>w. 412</i> ; .16, .31
148a	<i>w. 145 rev.</i> ; .38, .47
148b	<i>w. 145 rev.</i> ; .30, .46
149	<i>w. 145 rev.</i> ; — .36, — .51
149a	<i>w. 145 rev.</i> ; .23, .35
150	<i>w. 145 rev.</i> ; — .37, — .47
150a	<i>w. 146</i> ; — .73, — .74. <i>w. 145 rev.</i> ; .26, .43
150b	<i>w. 145 rev.</i> ; .22, .14
150c	<i>w. 145 rev.</i> ; .11, — .20
151	<i>w. 146</i> ; — .46, — .46. <i>w. 145 rev.</i> ; — .25, .31. <i>w. 130</i> ; — .25, — .21
152	<i>w. 146</i> ; — .51, — .30. <i>w. 145 rev.</i> ; — .08, .32. <i>w. 130</i> ; — .07, — .04. <i>w. 153</i> ; — .34, — .43
153	<i>w. 146</i> ; .03, — .02. <i>w. 124</i> ; .08, — .08. <i>w. 145 rev.</i> ; .29, .34, in 182 cities, .19.
154	<i>w. 146</i> ; — .22, — .35. <i>w. 124</i> ; — .44, — .48. <i>w. 145 rev.</i> ; .11, .28, in 182 cities, — .03
155	<i>w. 146</i> ; — .57, — .43. <i>w. 241</i> , — .37, — .39
156	<i>w. 146</i> ; .34, .33. <i>w. 145 rev.</i> ; .20, .24. <i>w. 130</i> ; .17, .27. <i>w. 124</i> ; .23, .24. <i>w. 144</i> ; .48, .44
157	<i>w. 146</i> ; .06, .03. <i>w. 150</i> ; — .18, — .11
158	<i>w. 146</i> ; .20, .20. <i>w. 130</i> ; — .03, .17
162	<i>w. 146</i> ; — .51, — .30. <i>w. 145 rev.</i> ; — .32, — .68. <i>w. 4</i> ; — .52, — .83. <i>w. 154</i> ; .06, — .14
163	<i>w. 146</i> ; — .49, — .36
164	<i>w. 146</i> ; — .62, — .62
165	<i>w. 146</i> ; — .31, — .41
166	<i>w. 146</i> ; — .19, — .22
167	<i>w. 146</i> ; — .44, — .44
168	<i>w. 146</i> ; — .60, — .34. <i>w. 145 rev.</i> ; — .24, — .44
169	<i>w. 146</i> ; — .32, — .22. <i>w. 145 rev.</i> ; — .37, — .66
171	<i>w. 146</i> ; — .11, — .23
172	<i>w. 146</i> ; .20, .04
173	<i>w. 146</i> ; — .54, — .56
174	<i>w. 146</i> ; — .60, — .56
177	<i>w. 146</i> ; — .37, — .46
179	<i>w. 146</i> ; — .40, — .43
180	<i>w. 146</i> ; — .43, — .44
181	<i>w. 146</i> ; — .51, — .53
182	<i>w. 146</i> ; — .41, — .47
183	<i>w. 146</i> ; — .48, — .44

TABLE 8—(continued)

Item	
184	<i>w. 146</i> ; -.50, -.52
185	<i>w. 146</i> ; -.25, -.05
186	<i>w. 146</i> ; -.45, -.40
187	<i>w. 146</i> ; -.57, -.68
188	<i>w. 145</i> ; -.51, -.51
189	<i>w. 146</i> ; .19, .06. <i>w. 145 rev.</i> ; .05, .19
190	<i>w. 146</i> ; -.41, -.47
191	<i>w. 146</i> ; -.09, -.11
194	<i>w. 146</i> ; -.51, -.35
197	<i>w. 146</i> ; -.49, -.46
201	<i>w. 146</i> ; -.26, -.36
207d	<i>w. 146</i> ; .19, .06. <i>w. 144</i> ; .28, .32
208	<i>w. 146</i> ; -.48, -.45. <i>w. 1</i> ; .37, .31
209	<i>w. 146</i> ; -.45, -.43. <i>w. 1</i> ; .28, .25
211	<i>w. 146</i> ; -.41, -.42
220	<i>w. 146</i> ; -.03, -.13. <i>w. 1</i> ; .19, .15
221	<i>w. 146</i> ; -.25, -.27. <i>w. 1</i> ; .51, .35
223	<i>w. 146</i> ; -.03, -.14. <i>w. 145 rev.</i> ; -.11, .12. <i>w. 1</i> ; .22, .11
241	<i>w. 146</i> ; .19, .07. <i>w. 124</i> ; .37, .23. <i>w. 145 rev.</i> ; .72, .76. <i>w. 242</i> ; .20, .27. <i>w. 134</i> ; .48, .66
242	<i>w. 145 rev.</i> ; .09, .18. <i>w. 155</i> ; -.37, -.39
243	<i>w. 145 rev.</i> ; .40, .47
244	<i>w. 124</i> ; .19, .06
249	<i>w. 146</i> ; -.18, -.26. <i>w. 124</i> ; -.19, -.17
251	<i>w. 146</i> ; -.17, -.12. <i>w. 124</i> ; -.03, .08. <i>w. 145 rev.</i> ; .16, .11. <i>w. 144</i> ; -.23, -.27. <i>w. 252</i> ; .76, .78. <i>w. 412</i> ; -.26, -.25
252	<i>w. 146</i> ; -.16, .03. <i>w. 145 rev.</i> ; .07, -.05. <i>w. 124</i> ; .17, .22. <i>w. 144</i> ; -.33, -.40. <i>w. 412</i> ; -.36, -.36
205	<i>w. 146</i> ; -.02, -.11. <i>w. 145 rev.</i> ; -.09, -.32
261	<i>w. 146</i> ; -.20, -.26
262	<i>w. 146</i> ; -.34, -.31
270	<i>w. 145 rev.</i> ; .24, .20
271	<i>w. 146</i> ; -.25, -.18
404	<i>w. 23</i> ; .60, .68. <i>w. 24</i> ; .65, .72. <i>w. 222</i> ; .58, .56. <i>w. 223</i> ; .40, .52
411	<i>w. 146</i> ; -.56, -.53. <i>w. 1</i> ; .32, .26. <i>w. 413</i> ; .31, .28
412	<i>w. 146</i> ; -.13, -.13. <i>w. 124</i> ; .28, .21. <i>w. 145 rev.</i> ; .49, .60. <i>w. 131</i> ; .51, .67. <i>w. 132</i> ; .37, .51. <i>w. 413</i> ; .37, .52. <i>w. 414</i> ; .39, .58. <i>w. 415</i> ; .49, .63
413	<i>w. 146</i> ; -.22, -.25. <i>w. 145 rev.</i> ; .29, .46. <i>w. 131</i> ; .27, .53. <i>w. 132</i> ; .06, .24. <i>w. 144</i> ; .22, .37. <i>w. 244</i> ; .00, .10. <i>w. 412</i> ; .37, .52. <i>w. 414</i> ; .19, .34. <i>w. 415</i> ; .23, .37
414	<i>w. 412</i> ; .39, .58. <i>w. 415</i> ; .49, .70
415	<i>w. 412</i> ; .49, .63. <i>w. 413</i> ; .23, .37. <i>w. 414</i> ; .49, .70
416	<i>w. 412</i> ; .09, .44. <i>w. 413</i> ; .28, .50. <i>w. 414</i> ; .54, .62. <i>w. 415</i> ; .37, .55

A Note on Statistical Procedures

With very very few exceptions the items and combinations of items show continuous variation around a single mode. The distributions are often skewed, sometimes enormously skewed. My correlation coefficients, computed by $r = \cos \pi U$, are more comparable than Pearson r 's would be, but no one method can give strict comparability of all the correlations. The variations introduced by the treatment are not, I think, prejudicial in relation to any of the questions to answer which the correlations are used. Fortunately the distributions of G, I and P (shown in FIGURES 1 to 6) do not depart widely from the symmetrical, "normal" form.

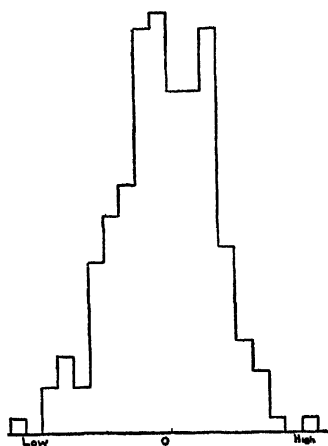


FIGURE 1. The distribution of G in the 200 cities.

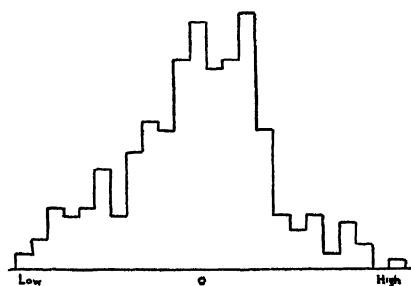


FIGURE 2. The distribution of G in the 295 cities.

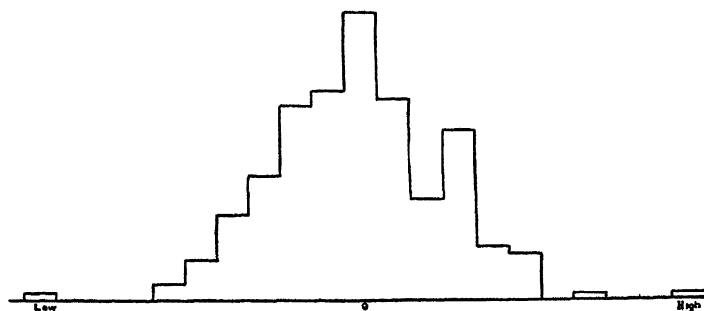


FIGURE 3. The distribution of I in the 200 cities.

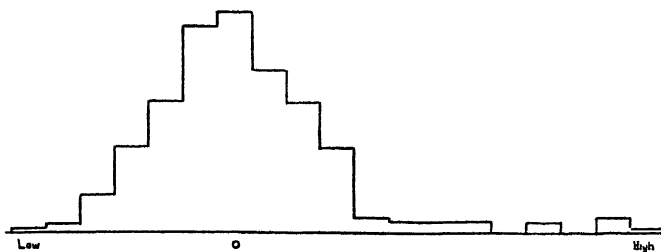


FIGURE 4. The distribution of I in the 295 cities.

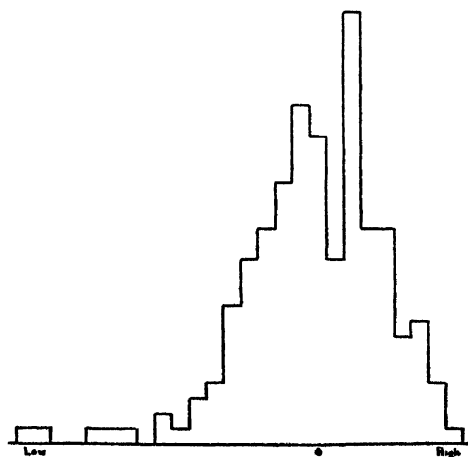


FIGURE 5. The distribution of P in the 200 cities.

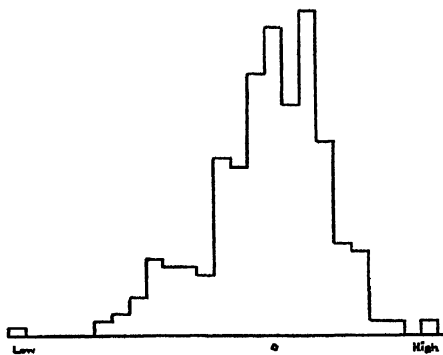


FIGURE 6. The distribution of P in the 295 cities.

The correlations computed from the facts for one or two or three years are somewhat lower (*i. e.* nearer to zero) than they would have been if computed from facts for more years representing more precisely the status of each city in comparison with the others. This "attenuation" varies from inappreciable amounts in the case of many of the items to .01 or more in the case of some. A correlation can be corrected for this attenuation by

$$r_{p\infty q\infty} = \frac{r_{pq}}{\sqrt{r_{pp_1} \cdot r_{qq_1}}}$$

in which $r_{p\infty q\infty}$ is the correlation which would be found if records from ten or more years (theoretically an infinite number of years) had been used, r_{pq} is the correlation obtained from the records actually used, r_{pp_1} is the correlation between the score actually used for one of the two items and a comparable score from another year or years, and r_{qq_1} is the same for the second item.

In applying this formula, r_{GG_1} , the correlation between our G score and a G score obtained in a similar manner from data for 1931, or for 1929, may be assumed to be over .99, since it is a composite of so many semi-independent items, and since most of them in most cities do not change suddenly with time. For the same reasons, r_{pp_1} may be assumed to be almost or quite .99. But r_{II_1} would be somewhat less stable from year to year, because an earthquake, a flood, the shutting down of a great mill and the like, could make a great change in I score in a few cities, and the special conditions of 1930 may have affected certain cities' incomes in special ways. I conjecture that r_{II_1} will be between .94 and .98, if data are ever available to compute it.

Whenever it was probable that a single year's record would not correlate with another year's to the extent of .90 or more, I have used records for two or more years (as many as six in the case of some items) if I could get them. But many items are obtainable for only a single year.

THE INFLUENCE OF THE DIFFERENCES BETWEEN COMMUNITIES IN THE PERCENTAGE OF THE POPULATION OF EACH SEX AND AGE

If the sex and age distribution was the same in every city, and the same in any one state as in any other state, the correlations reported in this monograph could be used unreservedly. Unfortunately for the workers in this field, cities vary considerably, and states appreciably,

in the proportions of male and female and of old and young. All the correlations involving per capita measures require consideration of the influence of these variations; and it will doubtless be desirable to have also many measures in which different weights are attached to different sex and age segments of the population. The correlations presented here are valuable as first approximations; and the per capita rate is better to use than any other one population rate. Also the variations among cities in many of the per capita items are so great that the influence of even large differences in, say, the percentage of children under 15 in the population may have a relatively minor influence upon the significance of say the per capita expenses for teachers' salaries, or ratio of dentists to lawyers, or other items which *a priori* seem specially sensitive to differences in the frequency of children per adult. But eventually the influence of all such sex and age differences should be measured and allowed for. They cannot be allowed for satisfactorily by partialling them out, because too much may be, and often would be, partialled out along with them. The differences in the number of females per hundred males act more simply, are less often influential, and are of smaller influence where they do operate, than the differences in age constitution. The influence of the latter is complex, acts in the case of almost all per capita measures, and may conceivably have a large influence upon some of them.

The following pages present measurements of the variations in sex and age distributions, and discussions of their influence as possible causes of imperfections in G, I, and P as measures to use in comparing cities, and of their significance in other ways. In considering correlations (especially those involving per capita measures) and conclusions drawn from them, the possibility of distortion by the "oldness" or "youthfulness" of a community should be kept in mind.

The variations in cities in the percentages of the population at ages 0-9, 10-14, 15-19, etc. are shown in Tables 9 and 10. They are large.

These variations are important in three ways: (1) as possible causes of imperfection in G, I, and P as measures to use in comparing cities, (2) as products of certain causes, and (3) as symptoms of certain conditions.

The Influence of Differences in Age-Composition upon the Validity of the G, I, and P scores

If three cities have the same G score, but vary in their age composition, having, respectively, few, an average number, and many of their populations under 15 years of age, will the general goodness of life for

good people old and young still be closely alike in all three? To answer this question we must consider the G score item by item.

Item 131, infant death rate reversed, is a rate based on live births, not a per capita rate. Hence the same score in it will mean the same percentage of absence of disappointment, grief, etc. to parents and friends. The same percentage will on the average be a larger per capita number in cities with many children under 15, since in such cities there will be more births per year per 1000 population, the correlation between the percentage under 15 and births per 1000 being about .40 (.43 in the 200 cities and .36 in the 295). In so far forth, the same score in Item 131 will represent more happiness per capita in the city where percent < 15 years is high. On the other hand, the loss of an only child or one of two may be a greater affliction than the loss of one out of four or five, and cities high in percent < 15 are also high in size of family (the correlations are .90 in the 200 and .78 in the 295 cities). The same score in Item 131, then, means perhaps a little more happiness or welfare per capita in the city with a high percent < 15.

Consider next item 53, per capita public expenditures for schools. The same per capita in our three cities will mean *fewer* dollars *per pupil* in the city with many children under 15, and so, other things being equal, inferior education and less welfare. This holds for items 54 and 55 also. The same score in Item 23, average salary of high-school teachers, however, will mean the same value in a city with many children as in one with few, in the sense that whoever are being educated are, so far as the item is concerned, being educated equally well. The item might properly be given more weight in a city with many children, but all are comparable in respect of it as a measure of the quality of education.

A given score in Item 57, per capita public expenditures for recreation, also seems to mean approximately equal contributions to welfare regardless of the age constitution of the population. Perhaps a young person gets more direct pleasure and health than an adult, so that a population having many children has a slight plus.

A given score in Item 107, the 5 percentile family rental, means a worse condition in a city with many children than in one with few. The item is the rent less than which is paid by a certain percent of the families in the city, not of its individuals, and, other things being equal, the more persons there are in a cottage or flat, renting from \$6 to \$20 a month, the worse it is for them and their neighbors.

The reverse is the case with the scores for gas, electricity, automo-

TABLE 9

THE VARIATION AMONG 295 CITIES IN THE PERCENTAGES OF THE POPULATION
AGED 0 TO 9, 10 TO 14, ETC.*

Per cents	0-9		10-14	Frequencies			65 or >	
	M	F	M + F	0-14 M + F	15-19 M + F	40-64 M F	M	F
0 to 0.9	—	—	—	—	—	—	—	1
1 " 1.9	—	—	—	—	—	—	—	4 4
2 " 2.9	—	—	—	—	—	—	—	23 12
3 " 3.9	—	—	—	—	—	—	—	69 37
4 " 4.9	—	—	—	—	—	—	—	52 61
5 " 5.9	—	—	—	—	—	—	—	76 54
6 " 6.9	—	—	—	—	—	—	—	45 67
7 " 7.9	—	—	—	—	—	—	—	17 33
8 " 8.9	—	—	—	—	—	—	—	5 10
9 " 9.9	—	1	—	—	—	—	—	2 7
10 " 10.9	—	1	—	—	—	—	—	1
11 " 11.9	—	4	—	—	—	—	—	—
12 " 12.9	2	5	3	—	—	—	—	—
13 " 13.9	10	15	7	—	4	—	2	— 1
14 " 14.9	12	24	30	—	11	—	—	—
15 " 15.9	28	48	38	—	27	—	1	—
16 " 16.9	37	69	63	—	83	1	3	—
17 " 17.9	69	38	58	—	53	2	3	—
18 " 18.9	59	40	40	2	65	4	5	—
19 " 19.9	37	28	26	5	25	—	8	—
20 " 20.9	23	10	14	6	18	3	12	—
21 " 21.9	12	6	6	7	7	10	30	—
22 " 22.9	4	2	6	20	—	20	24	—
23 " 23.9	1	2	1	28	2	27	24	—
24 " 24.9	1	2	1	43	—	42	35	—
25 " 25.9	—	—	1	36	—	38	31	—
26 " 26.9	—	—	—	41	—	46	35	—
27 " 27.9	—	—	—	36	—	33	32	—
28 " 28.9	—	—	—	32	—	32	21	—
29 " 29.9	—	—	—	12	—	16	18	—
30 " 30.9	—	—	—	12	—	11	4	—
31 " 31.9	—	—	—	8	—	2	3	—
32 " 32.9	—	—	—	4	—	6	2	—
33 " 33.9	—	—	—	2	—	1	1	—
34 " 34.9	—	—	—	—	—	—	—	—
35 " 35.9	—	—	—	—	—	1	—	—
36 " 36.9	—	—	—	1	—	—	1	—

* Some of the distributions given in Table 9 are computed by averaging the percentages of males and of females, at the ages in question, and so may differ very slightly from those which would be found by direct division. The differences are of no consequence, the nature and amount of the variation being almost identical regardless of the method of computation. The score used for per cent less than 15, for example, is really the average of the scores for per cent of males less than 15 and percent of females less than 15. It correlates practically perfectly with the percentage computed by dividing the sum of males under 15 and females under 15 by the total population (almost .995).

biles, telephones, and radios. These are all per capita scores and where there are more children and larger families, the percentage of persons who are benefited by them will be greater. This does not mean that one automobile for a family of six does as much good as

two automobiles for two families of three, or three automobiles for three families of two, but only that it does more good than one automobile for the two families or for the three families, with one or two of the families having none.

The same score in items 165 and 166, per capita deaths from syphilis (reversed) and homicide (reversed), will mean a worse condition in a

TABLE 10

THE VARIATION AMONG 295 CITIES IN THE PERCENTAGE OF MALES AT AGES 20-39,
AND IN THE PERCENTAGE OF FEMALES AT AGE 20-44

Percents*	Frequencies	
	Males 20-39	Females 20-44
28.....	4	
29.....	13	
30.....	26	
31.....	31	
32.....	47	
33.....	33	
34.....	37	
35.....	29	3
36.....	25	7
37.....	18	8
38.....	7	29
39.....	8	40
40.....	8	36
41.....	4	38
42.....	3	35
43.....	1	28
44.....	1	20
45.....		21
46.....		5
47.....		12
48.....		8
49.....		4
50.....		1

* 28 = 28.0 to 28.99, 29 = 29.0 to 29.99, etc.

city with many children than in one with few, since for the same per capita score, the frequency of syphilitics and murderers per 1000 persons over 15, which is the symptom we chiefly wish to measure, will be greater in the former than in the latter.

I have considered every item in G and conclude that in twelve items (134, 21, 22, 23, 24, 57, 17, 201, 202, 203, 26, and 11) a given score means the same merit or demerit regardless of the percentage of children,

and that the pluses and minuses among the others pretty nearly balance.

In the case of I, the index of per capita private income, the five income-tax, salary and wage components have the same meanings for I regardless of the percentage of children in the population. The expenditure items probably do not. The same average family rent will mean less income per capita if the families are large, as they will be if percent < 15 is large (but not less income per family). The same per capita expenditures for food will mean more income per capita because what a child 0 to 8 or older eats costs less than what an adult eats. The same per capita expenditures at cigar stores will mean more income per capita because what a child smokes costs less.*

The same per capita expenditures at drug stores will probably mean a little more income per capita, for the same reason. Food, cigar and drug expenditures had a total weight of 6 in our index; rent had a weight of 3. The other items had a total weight of 36. The imperfections in the index as a whole will then surely be very small, and it is not even certain that the same I score will mean less income for cities with many children. The error from rent may equal the opposite errors from food, tobacco, and drug-store sales.

In the case of P, the same per capita circulation of library books will mean more reading in a city with a high percentage at ages 0 to 14, unless the borrowers among children are specially numerous. The same per capita number of telephones will mean a larger percentage of adults supplying their families with telephones. The same per capita number of homes owned will mean a larger percentage of adults owning homes. On the other hand, the same per capita death-rates from syphilis and homicide will tend to mean worse conditions in cities with many children under 15. The significance (for the intellectual and moral status of the population) of a given score in the other constituents of P will be closely the same regardless of the percentage of children under 15. On the whole, it would probably be well to scale down the P scores a little for cities with percentages under 26 at ages 0-14, and raise them a little for cities with percentages over 26. The improvement thereby made in the validity of the measures of the personal qualities of the population would not, however, be great. What I have done is to compute P scores corrected very generously by subtracting one seventh of a standard deviation in P for every

* Compute the income, for example, if we counted per capita income as 200 times the per capita sales of cigar stores, and had two populations which, age for age, smoked equally, but one of which was composed of 20,000 babies and 10,000 parents, whereas the other was composed of 10,000 babies and 20,000 parents.

standard deviation in per cent < 15 below 26 and adding one seventh of a standard deviation in P for every standard deviation in per cent < 15 above 26. This corrected P correlates .992 with the regular P. It would then be supererogatory to compute new P scores replacing certain per capita values by corresponding values per person 15 years old or over, per person 10 years old or over, and the like.

The Causation of Differences in the Constitution of Cities in Respect of Age

The cities with the highest percentages (30 to 37) of children under fifteen are: Bayonne, Bethlehem, Chicopee, Columbus (Ga.), Dearborn, East Chicago, El Paso, Fall River, Greensboro, Hammond, Hamtramck, Hazleton, High Point, Johnstown, Laredo, Lorain, McKeesport, New Britain, New Castle, Ogden, Perth Amboy, Port Arthur, Revere, West Allis, Wilkes-Barre, Winston-Salem, Woonsocket, and Youngstown. As a rule they are industrial cities and have large foreign-born populations.

Among possible causes of differences among cities in the percentage of the population under 15 we may consider the birth rate (per 1000 females aged 20-44), the percentage of foreign-born whites, the per capita church membership 13 or over, and the percentage of the population gainfully employed as workers in factories. The correlations of percent < 15 with these are, in order, .43, .34, .36, and .34 in the 200 cities, and .39, .23, .28, and .33 in the 295 cities. These together, however, account for only about a quarter of the variation in percent < 15 (30 percent of it in the 200 cities and 24 percent in the 295). The birth-rate is by far the most important of the four, having in fact more influence than all three of the others.

It may reasonably be assumed that much of the variation not accounted for by the birth rate is due to the desertion of certain cities by older people, and the attraction to these cities of families with young children from other cities, from the country, and from foreign lands. It would be reasonable for the older people who could afford to do so to desert cities low in G in favor of cities high in G. Children under fifteen are of course unable to do this. Migration is financially less feasible for parents having several children of that age. The less intelligent and less "moral" persons would presumably migrate less in the direction of high G scores. How much less is not known because the amount of such migration is not known.

The percentage of Negro families makes practically no difference to percent < 15 . The correlations are .05 and .015. The size of the city

has very little influence; the correlations with largeness are $-.09$ and $-.155$. The density of the population (persons per acre) has very little influence, the correlations being $-.04$ and $-.11$.

The percentage of the population aged 10 or over gainfully employed is correlated negatively with percent < 15 (for males, $-.465$ and $-.46$; for females, $-.38$ and $-.24$). Part of this antagonism would vanish if the percentages were of those aged 20 or over, or 18 or over. But the fact remains that the cities where employment is common are those where children are few. The correlations of percent < 15 with "persons per thousand out of a job and looking for a job" in 1930, and with "persons per thousand having jobs but laid off though able and willing to work" are all positive ($.065$, $.03$, $.20$, and $.20$ respectively), and would be higher if persons out of work per thousand *adults* had been used.

The Significance of the "Childness" and "Adulthood" of Cities

The correlations of various features of the age-distribution with G, I, and P are shown in Table 11. A separate monograph in which the facts now available would be supplemented by others would be required to elucidate fully the meaning of these. So far as I can now judge, the general facts will prove to be as follows: (1) parts, but only very small parts, of the positive correlations with what we may call "adulthood" are spurious, being due to unfairness of the indices themselves to cities varying from the average toward "childness." (2) Larger parts in the case of G, and especially in the case of I, are due to the difficulty of maintaining a good life for citizens and at the same time caring for many children, and to the very small contributions of children to per capita income. (3) There remain large parts, especially in the case of G and P, which testify to a genuine tendency of those cities which are high in G because they are high in P or in what is common to P and I to have fewer children born and to attract and retain families with fewer children.

Evidence of the existence and magnitude of this third cause of the correlations is found in such facts as the following:* The antagonism of "childness" to P is greater than to I ($-.485$ compared with $-.35$) although the first two sorts of influence should make the latter much larger. The antagonism of "childness" to G after equalization for differences in I is greater than after equalization for differences in P ($-.28$ compared with $-.14$) although the first two sorts of influence should make the latter correlation the larger. The antagonism of

* These facts need to be considered in the light of their affiliations.

"childness" to P after equalization for differences in I is substantial and greater than its antagonism to I after equalization for differences in P ($-.47$ compared with $-.34$). The first two sorts of influence should produce a contrary effect.

The antagonism of childness to the per capita number of artists, musicians, professional workers other than teachers, and other persons who are frequent in "good" cities is much greater than its antagonism

TABLE 11

THE CORRELATIONS OF THE PERCENTAGES OF THE POPULATION OF AGE 0 TO 9, 10 TO 14, ETC., WITH G, I, AND P

	With G		With I		With P	
	200 cities	295 cities	200 cities	295 cities	200 cities	295 cities
Percentage at age 0-9, Male.	-.61	-.53	-.625	-.56	-.35	-.46
Percentage at age 0-9, Female.	-.43	-.43	-.36	-.44	-.47	-.49
Percentage at age 0-9, All..	-.44	-.41	-.43	-.47	-.41	-.44
Percentage at age 0-14, All.	-.45	-.42	-.29	-.41	-.48	-.49
Percentage at age 10-14, All.	-.32	-.32	-.09	-.23	-.38	-.40
Percentage at age 15-19, All..	-.34	-.33	-.08	-.36	-.40	-.58
Percentage at age 20-39, Male	.16	.05	.34	.21	.11	-.33
Percentage at age 20-44, Female	.11	-.07	.31	.09	-.08	-.24
Percentage at age 40-64, Male .	.40	.41	.26	.43	.37	.57
Percentage at age 40-64, Female.	.31	.46	.11	.44	.40	.64
Percentage at age 65 or >, Male..	.31	.37	-.205	.05	.61	.66
Percentage at age 65 or >, Female	.24	.40	-.02	.27	.49	.64
Percentage at age 65 or >, All...	.28	.33	-.085	.12	.57	.61

to the per capita number of policemen, public-service employees and other persons who are as frequent in "bad" cities as in "good." Sample facts are shown below. The influence of the mere dilution of the adult population capable of counting as artists, musicians, policemen, etc., by high percentages under age 15 would be to make these two groups of correlations about equally negative.

	200 cities	295 cities
male artists	-.48	-.44
female artists	-.50	-.52
The correlation of per cent < 15 with per capita number of		
male musicians	-.54	-.56
female musicians	-.60	-.56
female physicians	-.31	-.41
male dentists	-.37	-.46
male professional except teachers	-.57	-.68
female professional except teachers	-.51	-.51
The average of these eight correlations	-.49	-.52
male domestic servants	-.50	-.34
The correlation of per cent < 15 with per capita number of		
female domestic servants	-.32	-.22
policemen	.19	.06
male public-service employees	-.25	-.05
female public-service employees	-.45	-.44
The average of the last five correlations	-.27	-.20

The correlations of Boy Scouts per capita (*i. e.* of the percentage of the total population who are members of Boy Scout troupes) with G, I, P and percent < 15 are respectively .56, .58, .36 and - .22 (averages of .54 and .58, .57 and .59, .26 and .45, and - .18 and - .26). In this case the use of "per the total population" instead of "per the number admissible to Boy Scout troupes" tends to make the correlation unduly high. Consequently, if per cent < 15 did not have a substantial intrinsic antagonism to goodness in cities and in their populations it would correlate positively and fairly high with Boy Scouts per capita, and its correlation with Boy Scouts per capita would be little altered by equalization for G. Actually its correlations with percent < 15 are - .18 and - .26 and are raised to + .08 and - .02 by the equalization.

We may obtain an approximate measure of the average antagonism of percent < 15 to "good" traits apart from the influence of the mere replacement of old by young in the population, from correlations with the infant-death-rate (reversed), the percentage which the persons 16 and 17 years old in school are of all those of that age, average teachers' salaries, the infrequency of child labor, and other items which are not taken per capita. For items 2, 17, 18, 19, 21, 201, 202, 213, 214, 238, and 239 the median correlations with percent < 15 are - .22 for the 200 cities and - .28 for the 295.

A useful approach to the problems of allowance for the age constitution of a community in drawing inferences from the correlations of per capita items is to compute not only the per capita measure but also that per person < 20 years of age, and that per person 20 or over. If the last two correlate very closely, one with the other, we know that the differences of the communities apart from age constitution are so much more important than those due to it that the latter may be neglected with a little or no resulting error.

I have made such computations for some of the measures in the case of the states, where the positive correlations of "oldness" with G, I and P are closer than in cities and the negative correlations of "youthfulness" are more negative. The results are stated below, arranged in order according to the closeness of the correlation, which is in every case between the number of the thing per thousand persons 20 or over in the state and the number per thousand persons under 20.

$r = .98$ or higher	Dentists, M
Installations of gas	Osteopaths, F
Church membership, Unitarian,	Technical Engineers, M
Universalist and Christian	$r = .92$ to .939
Scientist	Graduates from high-school in a
Church membership, Roman	recent year
Catholic	Domestic servants, M
Physicians, F	Lawyers, M
Musicians, M	Musicians, F
Designers, F	$r = .85$ to .89
Osteopaths, M	Domestic servants, F
Dentists, F	Married women employed
Nurses, M	$r = .70$ to .74
$r = .96$ to .979	Homes owned
Telephones	Physicians, M
Radios	Teachers, M
Wired homes	$r = .61$ Church membership (all)
Church membership, Jewish	$r = .59$ Teachers, F
Clergy, F	$r = .38$ Clergy, M
Nurses, F	

It is evident that in all save eight of the above the correlations will be closely the same whatever weight is attached to the residents under 20 in comparison with those over 20. In the case of the eight, the actual correlations of G, I and P have been computed with the measures of per 100 under 20 and per hundred 20 or over. The corresponding per capita correlations from Table 7 are reported here for comparison.

	G			I			P		
	< 20	all	> 20	< 20	all	> 20	< 20	all	> 20
Female domestic servants.....	-.26	-.61	-.50	.26	.00	-.13	-.26	-.50	-.38
Married women employed.....	-.13	-.26	-.50	.61	.38	.13	-.38	-.38	-.50
Homes owned.....	.61	.50	.38	.00	.50	.61	.71	.71	.79
Male physicians.....	.38	.44	.00	.71	.44	.26	.38	.44	.00
Male teachers.....	.61	.38	.26	.26	-.03	-.13	.61	.38	.13
Church membership, all.....	.26 [*]	-.13	-.13 [*]	.38 [*]	.20	-.26 [*]	.13 [*]	-.20	.00 [*]
Female teachers.....	.81	.61	.13	.51	.26	-.26	.87	.86	.50
Male clergy.....	.13	-.38	-.50	-.13	-.61	-.86	.38	-.13	-.61

* Approximately, using 1930 populations instead of the means of 1920 and 1930 populations.

On the whole it seems that the conclusions based on per capita correlations for states would not be much modified by any reasonable considerations of the age distribution in states.†

† Since this monograph was accepted for publication I have been able to compute the correlations for the 295 cities between the number of radios per thousand of the population under age 20 and the number of radios per thousand of the population 20 or over, and similarly for thirty-four other important items. Fourteen of the 35 correlations are .98 or higher; 5 are from .96 to .979, 6 are from .92 to .959; 8 are from .85 to .919. One is at .81 and one at .76.

The only differences over .25 between the *G* correlations per capita and the per 100 aged 20 or over are for male physicians and female teachers, whose frequency is less affiliated with welfare when the young are left out of account. But obviously they should *not* be left out of account in considering these items. The same holds for the correlations of the frequency of physicians and teachers with *P*. The poorer showing of the frequency of male clergymen when only the adult population is used supports the conclusion that in communities with identical age constitutions, the frequency of clergymen would correlate negatively with welfare, income and personal qualities.

CAUSATION

Variation in *G* Scores Determined by Factors in *I* and *P*

The per capita amount of taxable property (call it *W*) is positively related to *G*, but only by its influence upon *I*.

Let *W* = a city's score in per capita taxable property

Let *Wr* = a city's score in per capita taxable real estate

Let *I* = a city's score in per capita taxable private income

Let *G* = a city's score in the general goodness of life for good people

Use the customary notations $r_{GW.I}$ and $r_{GWr.I}$ for the correlations between *G* and *W* and between *G* and *Wr* in cities identical in *I*.

Use the formula for partial correlation $r_{AB.C} = \frac{r_{AB} - (r_{AC})(r_{BC})}{\sqrt{(1-r_{AC}^2)(1-r_{BC}^2)}}$

The facts for the 200 cities and for the 295 cities are then:

	200 cities	295 cities		200 cities	295 cities
r_{GW} =	.28	.33	r_{GWr} =	.27	.43
r_{WI} =	.51	.48	r_{WrI} =	.59	.61
r_{GI} =	.525	.70	r_{GI} =	.525	.70
$r_{GIV.I}$ =	.01	-.015	$r_{GWr.I}$ =	-.06	.01

The variation in *G* in cities is largely determined by *I* and *P*, the correlations and the percentages determined by what is peculiar to *I*, what is peculiar to *P*, and what *I* and *P* have in common being as shown below.

CORRELATIONS

	200 CITIES				295 CITIES			
	Pearson	Cosine	πU	Average	Pearson	Cosine	πU	Average
I with <i>G</i>595	.525		.56	.74	.70		.72
P with <i>G</i>70	.68		.69	.84	.76		.80
I with <i>P</i>19	-.05		.07	.48	.30		.39

PERCENTAGES OF THE VARIATIONS OF CITIES IN G ACCOUNTED FOR BY THEIR
I AND P SCORES, AS CALCULATED BY PATH COEFFICIENTS

	200 cities	295 cities
By components of I not related to P.....	.265	.23
By components of P not related to I.....	.43	.375
By components common to I and P.....	.045	.23
Forces other than those influencing W, I or P.....	.26	.165

W acts only by what it has in common with I, contributing nothing by itself alone.

For the 48 states the correlations and determinations are:

Correlations	Pearson	Cosine PU	Average
I with G.....	.735	.61	.67
P with G.....	.87	.83	.85
I with P.....	.50	.38	.44

Percentages of variation in G determined by:

Components of I alone.....	.16	.12	.14
Components of P alone.....	.45	.47	.46
Components common to I and P.....	.27	.20	.235
All.....	.12	.21	.165

Factors represented by I, P and whatever I and P measure in common account for 74% of the variation in the goodness of life as measured by G in the 200 cities, 83½% in the 295 cities, and 83½% in the 48 states.*

G scores without these nine measures have the following correlations:

* The reader may wonder whether the inclusion in G of some of the items constituting I and P may not have had an improper influence in elevating the correlations of G with I and P. The items in question are average salaries of high school teachers, of elementary school teachers and factory workers (used for both G and I), and frequency of home ownership and rent of telephones, infrequency of illiteracy and of deaths from syphilis and homicide, and prevalence of doctors and trained nurses rather than male domestic servants (used for both G and P). These inclusions may elevate the correlations, but not improperly if they are genuine symptoms of the general goodness of life for good people as well as of per capita income and desirable personal qualities in the population. The only questionable one is the inclusion in P of per capita number of telephones. It was put in P as a symptom of intelligent care for the family and the convenience of friends, and belongs there. However, I knew that it correlated high with G and low with I, and may have been prejudiced by this knowledge and have given it too much weight.

For the sake of readers who wish to know what the correlations would be for a G which included no I or P items, I have computed G scores without them. The correlations with I and P are shown below, but, as has just been stated, it would be wrong to use these in place of those given above. Cities which have higher wages for teachers and factory workers, higher scores for home ownership, fewer murders and syphilitics, etc. do have in so far forth a better life for good people. To exclude such items from G because they elevated the correlations would be folly.

	Pearson	Cosine πU	Average
With I in the 200 cities.59	.56	.575
With I in the 295 cities.71	.70	.705
With I in the 48 states.785	.71	.75
With P in the 200 cities.61	.64	.625
With P in the 295 cities.78	.705	.74
With P in the 48 states.87	.83	.85

Factors Determining G other than by Affiliation with I and P

The next problem is to find any features of cities which influence their G scores other than by affiliation with I and P.

The procedure is to compute the percentage of the variation among cities in G due to I + P, then that due to I + P + the feature in question. The influence of the feature independently of I and P is obtained by subtracting the former from the latter. For various reasons, these determinations on the basis of only 200 or 295 cities are not highly reliable, but they are infinitely superior to guesses.

If certain features are found to contribute thus, independently of P and I, we have the further task of determining how much each contributes independently of the others. As the number increases, the labor of doing this becomes almost unendurable, and when the contribution seems likely to be less than one-half of one percent, I have not undertaken it.

In computing the percentage of the variation in G due to I + P, to I + P + P_1 (infrequency of illegitimate births and deaths from venereal infections other than syphilis), to I + P + Gov., to I + P + fewness of Negroes, etc., I have, as a rule, used Sewall Wright's method of path coefficients. It has the merit of allotting the causation in each case to what each cause has independent of the others, and to what each pair of causes have in common.

Using correlations computed by Sheppard's formula and shown in Table 7, we find the facts of Table 12.

These additions are divided clearly into two groups, five being .08 or more, and five being .02 or less. The first five (P, the composite of 11 and 12, Gov., the physical health composite, and the percentage of foreign-born whites) deserve, on the basis of Table 12, to be tried out in multiple correlations.

The intercorrelations of G, I, P, P_1 , 11 + 12, Gov., "Health," and "Foreign" are as shown in Table 13. The multiple correlations and percentages of the variation in G in the 295 cities accounted for by P, I + P, I + P + P_1 , I + P + P_1 + 11 + 12, etc., are as follows:

	Multiple Correlations with G	Percentages of G de- termined	Successive increments
P.....	.76	.5776	
I + P.....	.90134	.8124	.235
I + P + P ₁92945	.8639	.05
I + P + P ₁ + 11 + 12.....	.93573	.8756	.01
I + P + P ₁ + 11 + 12 + Gov.....	.94213	.8876	.01
I + P + P ₁ + 11 + 12 + Gov. + "Health".....	.95293	.9081	.02
I + P + P ₁ + 11 + 12 + Gov. + "Health" + "Foreign".....	.95741	.9166	.01

TABLE 12

PERCENTAGE OF THE VARIATION AMONG 295 CITIES IN G DETERMINED BY EACH OF
VARIOUS ADDITIONS TO I + P

P ₁ (composite of per capita illegitimate births and deaths from venereal diseases other than syphilis).....	.08
I + P in the past (composite of 11 and 12).....	.10
Gov. (composite of item 270 [approved features], typhoid rate reversed, and homicide rate reversed).....	.11
Physical health (composite of general death-rate reversed and infant death-rate reversed).....	.12
Fewness of Negroes.....	.02
Fewness of children under fifteen years.....	.01
Equality in incomes as evidenced by amounts paid for rent (or equivalent, if home is owned).....	.00
Per capita number of men eminent in art, literature, science and affairs....	.01
Number of inhabitants per acre.....	-.01
Percentage of foreign-born whites.....	.08

TABLE 13

THE INTERCORRELATIONS OF G, P, I, P₁, 11 + 12, Gov., "HEALTH," AND 144
(PER CENT OF FOREIGN-BORN WHITES)

For the 295 cities

	P	I	P ₁	11+12	Gov.	"Health"	144
G.....	.76	.70	.60	.72	.69	.71	.51
P.....30	.32	.66	.39	.43	.21
I.....44	.46	.57	.50	.50
P ₁34	.58	.70	.68
11+12.....525	.37	.37
Gov. (134+241+271).....63	.66
"Health" (131+132).....69
144 (% foreign-born white)...

The enrichment of P by the items of illegitimate births and deaths from certain venereal diseases has thus a substantial influence, and there is evidence that if we could obtain adequate measures of the P of the past generation, the quality of the municipal government, and the physical constitution of the residents, some or all of these would have a substantial influence.

Both of these conclusions are supported by the multiple correlations for the 200 cities which remain when suburban cities and cities of the Old South are omitted. The intercorrelations are as shown in Table 14. The percentages of G determined by P, I + P, I + P + P₁, etc., and the successive increments for the 200 cities are as follows:

	Percentages of G deter- mined	Successive Increments
P.....	.4624	
I+P.....	.7041	.24
I+P+P ₁7756	.07
I+P+P ₁ +11+12.....	.8055	.03
I+P+P ₁ +11+12+Gov.....	.8484	.04
I+P+P ₁ +11+12+Gov.+ "Health".....	.8548	.005

Some statisticians among my readers may be sceptical concerning

TABLE 14
THE INTERCORRELATIONS OF G, P, I, P₁, 11+12, Gov., "HEALTH", AND 144
For the 200 cities

	P	I	P ₁	11+12	Gov.	"Health"	144
G.....	.68	.525	.37	.66	.51	.56	.26
P.....	...	-.05	-.07	.465	.03	.28	-.16
I.....12	.29	.38	.28	.32
P ₁19	.39	.49	.53
11+12.....37	.23	.22
Gov.....495	.51
"Health" (131+132).....48
144 (% foreign-born white).....

the computation of path coefficients and multiple correlations from correlation coefficients computed by Sheppard's formula. I have therefore computed Pearson coefficients for the intercorrelation of G, I, P, P₁, 11 + 12, Gov., and "Health," after first shrinking up the skewed ends of the distributions until each is approximately symmetrical and 'normal.' The chief member G requires no adjustment; P requires only a shrinking from 4 units to 3 beginning a little below the median, and then of 5 units to 3. In I there is a progressive shrinking

of plus scores, making thirty-six scale units equal to eighteen on the minus side. In P_1 there is a large progressive shrinking of the minus scores (*i. e.* high illegitimacies and high death-rates) about one hundred and fifty scale units being made equal to eighteen. In 11 + 12, fifty-two scale units on the plus side are made equal to twenty-two on the minus side. In Gov., 170 minus scale units are made equal to 65 pluses. In "Health," 60 minus scale units are made equal to 30 pluses.

The intercorrelations found by computing Pearson coefficients for these modified scores are presented in TABLES 15 and 16. The multiple correlations and percentages of G determined are shown below

	Multiple Correlations with G	Percentages of G deter- mined	Successive Increments
P.....	.825	.6806	
I+P.....	.92564	.8568	.16
I+P+ P_193550	.8752	.02
I+P+ P_1 +11+12.....	.94488	.8928	.015
I+P+ P_1 +11+12+Gov.....	.94990	.9023	.01
I+P+ P_1 +11+12+Gov.+ "Health"....	.95781	.9174	.015

TABLE 15

THE INTERCORRELATIONS OF G, P, I, P_1 , 11+12, Gov., AND "HEALTH" IN THE 200 CITIES USING PEARSON COEFFICIENTS COMPUTED FROM MODIFIED DISTRIBUTIONS

	P	I	P_1	11+12	Gov.	"Health"
G.....	.71	.595	.29	.58	.455	.47
P.....15	.12	.455	.17	.22
I.....155	.245	.295	.26
P_108	.33	.425
11+12.....175	.04
Gov.....40
"Health".....

TABLE 16

THE INTERCORRELATIONS OF G, P, I, P_1 , 11+12, Gov., AND "HEALTH" IN THE 295 CITIES USING PEARSON COEFFICIENTS COMPUTED FROM MODIFIED DISTRIBUTIONS

	P	I	P_1	11+12	Gov.	"Health"
G.....	.825	.776	.630	.640	.634	.670
P.....500	.462	.539	.439	.477
I.....514	.456	.500	.500
P_1300	.520	.696
11+12..457	.246
Gov.....549

(for the 295 cities only). The discrepancies are of about the magnitude to be expected from the data, and do not alter the general conclusions stated above.

The main conclusion, that the personal qualities of the populations ($P + P_1$) and their incomes account for most of the variation of cities in G , holds good by any reasonable method whatever of measuring the correlations. For example, if Pearson coefficients are computed using the unmodified scores (deviations being taken from the median) the percentage of G determined by $P + I$ is .856 for the 295 cities, and .711 for the 200 cities. If one uses the average of such a Pearson coefficient and the Sheppard coefficient, the corresponding figures are .874 and .740. If one computes r from the variabilities of the arrays, or from the observed departure of the regression line from the line which would be found if the highest city in G were the highest in P and so on, similar results will be found.

THE INFLUENCE UPON WELFARE OF VARIOUS FEATURES OF THE LIFE OF A CITY OR STATE

Finally we may consider the causal influence, or at least the symptomatic value, of such features as rapidity of recent growth, immigration from foreign lands, the frequency of certain occupational groups, the frequency of specially able persons, child labor, and the predominance of factories, regardless of whether these are influential upon welfare only indirectly via their influence upon income and personal qualities. Some of the facts for the cities have been published elsewhere.* These will be repeated here only when they are desirable introductions to the new material.

Rapidity of Recent Growth

Rapidity of recent growth (1900 to 1930) had a very slight positive relation with G (.065), a small positive relation (.175) with I , and a very slight negative relation with P ($-.065$). For the states these correlations are .43, .36 and .10 respectively. The "good" states have grown faster than the "bad" states by immigration and lower death-rates, not by higher birth rates. They have in fact smaller percentages of the population under 15.

Sex

The number of males per 100 females varies more widely than common experience supposes—from 95 in Massachusetts and Rhode

* *Your City*, by E. L. Thorndike, especially Chapter V.

Island to 120 in Montana, 123 in Wyoming and 140 in Nevada. It is higher in the states high in G and P, but is very little higher than average in the states high in I. The correlations with G, I and P are .61, .13 and .61, whereas they were .15, .24 and .01 for cities. The correlations for states are rather a mystery. Perhaps males are freer to move long distances when attracted by better conditions. Perhaps it is a matter of chance. The probable error of the .61 is .20.

Race and Nationality

The percentage of Negroes has had affiliations with hardly an exception, but its correlations with Income are much less negative than its correlations with G and P, being .02 in the 200 cities and — .25 in the 295 cities.

The facts for the states are in general the same as those for cities. The correlations are as follows:

	With G	With I	With P
% Native-born whites.....	.19	— .19	.32
% Foreign-born whites.....	.78	.82	.82
% Negroes.....	— .61	— .50	— .79
% Negroes and other colored.....	— .79	— .38	— .925

When the percentage of Negroes is partialled out the correlations are as follows:

	With G	With I	With P
Percentage native-born whites in 200 cities.....	.34	— .17	.635
Percentage native-born whites in 295 cities.....	.275	.03	.61
Percentage native-born whites in 48 states.....	.13	— .30	.35
Percentage foreign-born whites in 200 cities.....	.05	.47	— .46
Percentage foreign-born whites in 295 cities.....	.245	.45	— .21
Percentage foreign-born whites in 48 states.....	.66	.84	.48

When the percentage of Negroes and other colored is partialled out, the correlations for the states are:

	With G	With I	With P
% Native-born whites.....	.14	— .26	.53
% Foreign-born whites.....	.36	.98	.24

These correlations are all computed by cosine πU . If the distributions for the percentage of Negroes and for the percentage of all colored are made to approximate the "normal" form by multiplying each minus deviation from the median by 2 and each plus deviation by 0.5, and Pearson coefficients are then calculated, the correlations in states are:

	With G	With I	With P
% Native-born whites.....	.19	-.13	.46
% Foreign-born whites.....	.68	.75	.58
% Negroes.....	-.83	-.53	-.88
% Negroes and other colored....	-.75	-.47	-.83

When the percentage of Negroes is partialled out, the correlations for the states are:

	With G	With I	With P
% Native-born whites.....	.29	-.46	.21
% Foreign-born whites.....	.42	.64	.16

When the percentage of Negroes and other colored is partialled out, they are:

	With G	With I	With P
% Native-born whites.....	-.12	-.36	.31
% Foreign-born whites.....	.42	.70	.16

The central tendencies of the four partial correlations for the states (by the two methods and with use of Negroes and of all colored) are as follows (Zeta transformations were used in this case):—

	With G	With I	With P
Native-born whites.....	.11	-.36	.36
Foreign-born whites.....	.47	.86	.27

As compared with the native-born, after equalization for the percentage of Negroes, the foreign-born are found especially in the "better" and richer states, and in the richer cities with inferior populations.

Some of the relevant correlations of the percentage of foreign-born white families are appended, but the reader should remember that the merits and demerits of the foreign-born will be much more satisfactorily measured by studying individuals than by studying cities.

Correlation of the percentage of foreign-born whites:

	In the 200 cities	In the 295 cities
With percentage of the population under 15 years.....	.34	.23
With fewness of Negroes.....	.465	.58
With births per 1000 females 20-44 years.....	.15	-.01
With per capita number of factory workers.....	.48	.44
With per capita number of church members 13 or over....	.50	.25
With infant death-rate (reversed).....	.525	.67
With general death-rate (reversed).....	.31	.58
With appendicitis death-rate (reversed).....	.38	.58
With puerperal disease death-rate (reversed).....	.34	.63

Occupational Groups

Dentists, designers, artists, engineers, musicians, architects, trained nurses (female), and osteopaths are found in large numbers in the good cities, which have relatively few lawyers, male nurses, veterinaries, clergymen, and domestic servants. The states show the same except that lawyers, veterinaries and woman teachers move up while artists move down.

The correlations of the frequencies of certain occupational groups in cities with I and P (indices of income and desirable personal qualities of intellect and character) were instructive. Dentists, trained nurses, male teachers and male musicians were not specially frequent in rich cities, but were so in cities scoring high in P. Male artists, architects, designers and engineers, on the contrary, are much more frequent in rich cities than in cities high in P. In states the second half of the comparison holds true, but the first does not. The facts are shown in TABLE 17.

TABLE 17

THE ASSOCIATION OF CERTAIN OCCUPATIONS WITH THE WEALTH AND PERSONAL QUALITIES OF A POPULATION

	In Cities			In States		
	Correlations		Difference in favor of I	Correlations		Difference in favor of I
	With I	With P		With I	With P	
Dentists (male) ..	.265	.705	— 44	.79	.70	.09
Trained nurses (female) .	.145	.305	— .16	.83	.55	.28
Male musicians	.065	.565	— 50	.955	.39	.56
Male teachers	.14	.51	— 37	— .03	.38	— 41
Artists (male)	.48	.255	225	.925	.26	.665
Architects (male)	.445	.05	395	.885	.03	.855
Designers (male) .	.635	.165	47	.87	.39	.48
Engineers (male)	.49	.18	31	.965	.26	.705

Eminent Men

The percentage of the population listed in Who's Who and the percentage enrolled in the American Association for the Advancement of Science have very low correlations with G and P in cities, but in states they are substantial, the fact being as follows:

Who's Who	with G: in cities	.09,	in states	.23
A. A. A. S.	with G: in cities	.175,	in states	.45
Who's Who	with P: in cities	.045,	in states	.30
A. A. A. S.	with P: in cities	.06,	in states	.33

The relations to per capita income are everywhere closer. Eminent artists, literary men, scientists, and the like are rarely produced, still less supported, in places of poverty. The correlations with I are .14 and .275 for cities, .86 and .93 for states. The percentage of A. A. S. members ranks along with average salary of clerks, retail sales, service, hotel and amusement receipts, frequency of professional workers, and frequency of artists as a symptom of the per capita income of a state, and is clearly surpassed only by the salaries of teachers and the percentage of income-tax returns.

Women in the Professions

States differ widely in the relative frequency of men and women in the professions. These $\frac{F}{M}$ ratios are practically unrelated to I or P in cities (the medians of sixteen such with I being .00; and with P, .025). There is a very slight feminist tendency in the better cities, the median of the sixteen correlations with G being .08. The median correlations for states are .36 with G, .30 with I, and .265 with P, all representing probably a tendency of the states superior in the goodness of life, income and personnel to be willing to try new ways. The correlations with the per capita number of osteopaths in the states are of interest in this connection. They are .42 with G, .405 with I and .605 with P, and are subject to little or no reduction by allowances for differences in the age distribution.

Employment

The gainful employment of married women, of boys under 15 and of girls under 15 is a bad sign for a city or a state. The order of demerit is as given, the employment of girls being the worst.

The correlations are:

	With G		With P	
	Cities	States	Cities	States
Married Women	-.10	-.26	-.01	-.38
Boys under 15.	-.535	-.66	-.425	-.555
Girls under 15	-.585	-.79	-.69	-.865

It would indeed be entirely justifiable to include the last (reversed) in an extended P index, as well as in the index of the general goodness of life.

Cities and states are able to get sufficient income in other ways than by working children. The richer ones work them least, the correlations with I being -.23 and -.66 for boys in cities and in states and

— .145 and — .13 for girls. The case of married women is not so clear, the correlations with I being — .08 in cities, but + .38 in states.

Manufacturing

I have shown elsewhere that the common diatribes against the factory city are largely unjustified, the correlations of percentage of the population employed as factory workers with G and P being slightly positive. The facts for the states confirm this. The correlations with G and P are both .10. Moreover, though the incomes of factory cities are only a bit above those of other cities, the incomes of factory states are much above those of other states, the correlation with I in states being .60. The presence of factory cities and towns means increased income for the farmers and other non-factory workers in the state.

Sales

Since differences in income are one large cause of the differences of states in welfare as measured by the G score, and since income and the intelligence and morality of the populations of the states are positively related, the correlation being .44 (.50 by Pearson and .38 by cosine πU), we must expect that sales of all sorts of things will be positively related to G and P via I. It is the differences in the closeness of the relation of, say, sales of bookstores and sales of drugstores to G and P that are most significant. So in addition to the actual correlations I report in Table 18 the difference of each from the median. It should be remembered that it is a property of correlation coefficients that a

TABLE 18

THE CORRELATIONS OF EIGHT SORTS OF PER CAPITA SALES WITH G, I AND P IN STATES, AND THEIR DIFFERENCES FROM THE MEDIAN OF THE EIGHT

	Correlations with			Differences from the median correlation		
	G	I	P	G	I	P
Cigar stores.....	.895	.83	.66	.395	-.02	.22
Music and radio stores.....	.79	.90	.60	.29	.05	.16
Food stores.....	.50	.925	.50	.00	.075	.06
Book stores.....	.54	.705	.65	.04	-.145	.21
All retail sales (1933).....	.50	.865	.38	.00	.015	-.06
Drug stores.....	.50	.61	.38	.00	-.24	-.06
Service, hotel, and amusements..	.50	.965	.26	.00	.115	-.18
Second-hand stores.....	.36	.75	-.17	-.14	-.10	-.61
Median.....	.50	.85	.44			

given difference up is really greater than the same difference down, and especially when the correlations approach 1.00.

Sales of cigar stores, music and radio stores and book stores are specially affiliated with G and P, but not with I. Sales of second hand stores and service, amusement and hotel receipts are weakly affiliated with G and P, but not with I. Sales of drug stores are weakly affiliated with G and P, and I also. The per capita sales of book stores are not available for cities. If, as is probably safe, we assume that their correlations with G, I and P in the 295 cities would be at least .20, .30 and .30, the facts for cities corresponding to these for states in TABLE 18 will be as shown in TABLE 19.

TABLE 19

THE DIFFERENCES OF THE CORRELATIONS OF EIGHT SORTS OF PER CAPITA SALES WITH G, I, AND P FROM THE MEDIANS FOR THE EIGHT, IN CITIES

	G	I	P
	median r	median r	median r
	= .13	= .23	= .22
Cigar stores.....	+.23	+.06	+.19
Music and radio stores.....	-.11	-.19	+.03
Food stores.....	+.31	+.42	+.19
Book stores.....	+ ?	+ ?	+ ?
All retail sales (1933).....	+.03	+.04	-.04
Drug stores.....	-.18	-.15	-.14
Service, hotels and amusements.....	-.03	-.05	-.16
Second-hand stores.....	-.40	-.28	-.38

These tables give a picture of the good community as neither puritanical nor miserly, spending little for drugs and cosmetics, staying at home with its books, magazines and radio rather than frequenting places of entertainment. This is confirmed by the facts concerning ownership of automobiles and radios. As in cities, so in states, it is not the ownership of the humbler cars that is significant of greater total welfare or of better people. The correlations with G and P are respectively .72 and .81 for "other than Ford," and only .50 and .61 for Ford cars. A radio in the home is better than electricity as a sign of G and P, the correlations being .25 and .05 higher.

The Home

As in cities so in states the number of homes owned per thousand population is indicative of G and P, the correlations being .50 and .71 respectively. The provision of electricity and of telephones in homes

is so also, the correlations with G being .50 and .63, and with P, .61 and .73.

Church Membership

From the data given in the 1926 Census of Religious Bodies we can compute the per capita membership (over 13 years of age) in all churches, in Jewish congregations, in Roman Catholic churches, and in a combination of Unitarians, Universalists, and Christian Scientists. The correlations in cities show the decline in the influence of the church upon welfare. For membership in all churches combined they are — .21 with G, and — .245 with P. Even with extreme allowance for possible influences of difference in the age distribution of the cities, they will still be clearly negative. This failure of church membership to be affiliated with welfare and desirable personal qualities is not confined to cities. For the states the corresponding correlations are — .13 and — .20. The Unitarians, Universalists, and Christian Scientists are an exception, the correlations for them being .51 and .52 in the cities, .61 and .71 in the states. They, however, are notably unorthodox, and opposite in many respects to the general temper of the church, so that the positive correlations for them may be taken to corroborate the conclusion from the negative correlations for the church as a whole.

The volume of membership in the Jewish congregations and in the Roman Catholic church may be in large measure a sign of a certain ancestry as well as of religious zeal. Consequently the correlations should be interpreted with caution. What we prefer to know of a city or state is the percentage of its Jewish population who are members of Jewish congregations and the percentage of its population from predominantly Catholic countries who are members of the Roman Catholic church. These facts are not available. Taking the membership percentages based on total population, the correlations are as follows:

	Cities		States	
	With G	With P	With G	With P
Jewish Congregations.	-.015	-.40	.26	.26
Roman Catholic Church.15	-.205	.30	.17

The Jewish and Catholic Churches have perhaps kept the allegiance of their better elements more than the Protestant denominations. A more penetrating analysis is required to decide this. The higher correlations may be caused by the confusion of race and religion in the statistics, by the infrequency of Negroes in these churches, by the

selective immigration into certain states, by the influence of income in determining a community's G score, and by other forces.

The correlations with I are all positive, being for the Jews, .38 in cities and .71 in states; and for the Catholics, .345 in cities and .70 in states. For all churches together they are low, .015 in cities and .20 in states. It has been shown that the foreign-born, who are a large fraction of the Jewish and Catholic memberships, have been attracted to and retained by the cities and states with higher incomes. They may also have had a substantial share in making these incomes high.

Other Social Institutions

The per capita membership in Kiwanis and that in Rotary are much alike in their affiliations and may be averaged. The correlations with G are slightly negative in cities, averaging $-.05$, and would be more negative after allowance for the larger percentages of adult males in the "better" cities. This antagonism is accounted for by the strength of these clubs in cities with low incomes (the average correlation with I is $-.24$); the correlations with P are positive (averaging .315). A large membership is characteristic of cities of good people with low incomes. As between states the case is somewhat different. All the correlations are higher. Those with G average .36; those with I average .03; those with P average .355. Membership in these clubs is more closely affiliated with the goodness of life for good people and with the intelligence and morality of the population (in so far as G and P scores parallel these qualities) and less closely affiliated with wealth than is membership in the churches other than the Unitarian, Universalist and Christian Science denominations.

The per capita membership of the Boy Scouts makes an enviable showing, its correlations with G and P being .555 and .355 in cities and .62 and .62 in states. Correction for the influence of differences in age distribution would raise these figures. That is, if the membership figures were not per capita, but per hundred persons aged 10 to 14, or 10 to 19, these correlations would be even higher. The correlations with I are .58 and .67.

It will be instructive to compare the essential figures for the per capita numbers of certain sorts of persons as shown below.

	Correlations			
	In cities		In states	
	with G	with P	with G	with P
High School graduating class of 1934*	.375	.645	.87	.93
Persons having radios80	.75	.61	.66
Persons having telephones685	.78	.63	.73
Unitarians, Universalists and Chris- tian Scientists51	.61	.52	.71
Boy Scouts*555	.355	.62	.62
Kiwanis and Rotary members	-.05	.315	.36	.355
Church members	-.21	-.245	-.13	-.20
Illiterates (aged 10 or over)	-.455	-.755	-.76	-.93

Disparity in Wealth and Income

The disparity, that is, the variability of the incomes of the families of the 295 cities studied can be measured roughly, but very instructively, by the disparity in the amounts paid for rent (or equivalent in the case of owned homes). It has been shown elsewhere (*Am. Jour. of Sociology*, 44: 25-35, 1938) that parity in incomes is correlated with G, but only in so far as it is a symptom of superior personal qualities in a population. Among cities identical in P score, parity in incomes has zero correlation with G.

A similar inquiry cannot be made for states because the value of the part of a farm property used as a home for the family and comparable to a house or apartment in a city has not been estimated and used in the census. One can get the reports for non-farm homes for the cities and towns above a certain size in each state, but it is unfair to compare states on the basis of these. Moreover many of the states report very large percentages undistributed save that all are below \$10 a month rent, or below \$1000 total value, with the result that the 5 percentile rental cannot be estimated even roughly, much less measured.

Birth Rate and Size of Family

In cities there is a very wide range (from below 3 to over 15) in the reported number of live births per thousand women aged 20 to 44. In 46 states (So. Dakota and Texas do not report the facts) there is a wide range, from 7.4 in California, 7.6 in Oregon and 7.9 in Washington to 16.6 in New Mexico. The human birth rate in this country is now very clearly a psychological and sociological rather than a physiological phenomenon. The correlation with G is negative

* These correlations would be somewhat higher if based on the frequencies in the age group concerned instead of on the frequencies in the total population.

($-.285$ in cities, $-.53$ in states). The correlation with P is $-.095$ in cities, $-.14$ in states. The correlation with I is $-.105$ in cities, $-.69$ in states. Among communities, as among individuals, the better have fewer children.

The correlations of median size of family in 1930 with G , I and P , which average (in that order) $-.18$, $-.01$ and $-.40$ for the two groups of cities, are much lower for the states, about $-.60$ in each case. If, as is reasonable, we may suppose that the states had closely the same relative positions in respect to welfare and personal qualities in 1910 as in 1930, the tendency of the "better" states to have smaller families has strengthened since then.

The Welfare of Cities in Relation to that of the Neighboring Country

There is a widespread notion that cities, especially large cities, gain wealth and welfare at the expense of the rest of the country. On the contrary, the wealth and welfare of a state as a whole is positively and closely related to that of the cities of 30,000 to 500,000 population within its boundaries. The correlations between the G , I or P score of each of the 42 states which have such cities and the median G , I or P score of such cities within it is $.97$ in the case of G , $.84$ in the case of I , and $.97$ in the case of P . If the able and good migrate from farms and villages to the cities, they probably advance the wealth and welfare of the state by doing so. At all events, one of the least promising procedures the farm and village residents of a state could take to benefit it would be to attack or thwart its cities.

MAY 13, 1940

KINETICS IN SOLUTION*

By

FRANK BRESCIA, HENRY EYRING, KEITH J. LAIDLER, VICTOR K. LAMER, SAMUEL H. MARON, IRVING ROBERTS, GEORGE SCATCHARD, J. C. WARNER, AND F. H. WESTHEIMER

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INTRODUCTION TO THE CONFERENCE ON KINETICS IN SOLUTION

BY VICTOR K. LAMER

From Columbia University, New York

The New York Academy of Sciences has recently inaugurated a series of conferences extending over a two day period upon topics of active interest in the field of physical chemistry.

This is the second in the series. The first conference dealt with Electrophoresis; the third with Dielectrics.

Chemical Kinetics has occupied the continued attention of a great many chemists since the birth of physical chemistry over half a century ago. The names of the pioneers: Wilhelmy, Harcourt and Esson, Warder, Van't Hoff, Ostwald and Arrhenius come at once to mind. Their fundamental researches dealt almost exclusively with the rates of chemical reactions in solution. Together with homogeneous and heterogeneous gas kinetics, chemical kinetics in some form or other constitutes the major field today of physical chemists in all parts of the world.

Owing to the greater technical difficulties of measurement, it is not until the dawn of the present century (1899 to be exact) that one encounters a homogeneous gas reaction studied as systematically and reliably as the solution reactions mentioned above. I refer, of course, to Bodenstein's classic investigation of the kinetics and equilibria in the gaseous system, hydrogen, iodine and hydrogen iodide.

Following the Great War, the investigation of homogeneous gas reactions has no longer been restricted to a very few laboratories but has become a very popular field of endeavor.

The recent interest in homogeneous gas reactions can be traced to several causes: to wit; the significant advances in the experimental techniques necessary for handling and measuring gaseous reactions at both high and low pressures, the failure to recognize the importance of chain reactions and free radicals in the early work, the wealth of pertinent information available from spectroscopy, the desire to reconcile unimolecular processes with collisional mechanisms and even to test the ill-fated radiation hypothesis.

Perhaps the most alluring stimulus has been the hope that by eliminating the solvent, the interpretation would become simpler and more amenable to unambiguous theoretical treatment. Unfortunately this wish has not been realized. It is indeed true that by eliminating

the solvent one can define more precisely what is meant by "a collision" or the "radius of influence" of a molecule or ion, and thus calculate the number of collisions between reactant molecules with much greater certainty than in the presence of a solvent. Unfortunately, the elimination of the solvent at the same time introduces many complications which often more than compensate for the advantages of its absence.

From the intensive study of homogeneous gas reactions over the past two decades, it now appears that far from complicating the situation, the presence of a solvent often simplifies the chemical process from a kinetic viewpoint. By maintaining a more uniform and a more rapid distribution of the energy liberated in the chemical reaction, the presence of a solvent often completely eliminates undesirable side reactions. Wall reactions are rarely of any consequence in solution and chain reactions can be readily recognized and interpreted.

The recognition of the difficulties of reproducing experimental results in gases at least to a precision sufficient to resolve moot points has caused a number of workers, who had previously been identified exclusively with gaseous kinetics to turn their attention within the last five years to solution kinetics. By bringing fresh points of view and experimental experience the kinetics in solution appear to be enjoying a renaissance. The analytical procedures available for solutions are so precise that the determination of energies of activation have now been reliably measured to ± 50 small calories for several reactions, whereas in gas reactions an energy of activation which is reproducible to 500 or even 1000 calories is considered exceedingly good.

For this reason many of the finer points in the theory of chemical kinetics, for example whether or not the energy of activation is temperature dependent, cannot be unambiguously clarified by appeal to such excellent data as those of Bodenstein on the hydrogen iodide reaction.

On the other hand, within the past six years, it has been demonstrated in more than one laboratory that the energy of activation in solution reactions is indeed temperature dependent and that an appreciable entropy of activation from this source must exist.

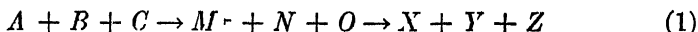
The papers fall into three groups. The first two deal primarily with the underlying theory of chemical kinetics as modified by the presence of a solvent; the next three with recent specific applications of isotopes; the last with applications to organic chemistry.

THE EFFECT OF SOLVENTS ON REACTION RATES¹

BY KLIFF J. LAIDLER² AND HENRY EYRING

From Princeton University, Princeton, N. J.

We propose to consider the influence of solvents on chemical reactions of the general type



in which the initial reactants, $A, B, C \dots$ give rise to the final products $X, Y, Z \dots$ with the intermediate formation of the activated complex M^{\ddagger} . $N, O \dots$, which we shall hereafter refer to as intermediates, are species which may be produced at the same time as the activated complex, and may also appear as final products of the reaction; in the denaturation of pepsin five hydrogen ions play this rôle. M^{\ddagger} differs from an ordinary molecule only in that it is unstable and has four degrees of translational freedom instead of the usual three, the extra one replacing the degree of vibrational freedom of the bond which is broken when the activated complex decomposes. In the application of the theory of absolute reaction rates⁴ the reaction is considered from the point of view of an equilibrium between the reacting species $A, B, C \dots$ on the one hand, and $M^{\ddagger}, N, O \dots$ on the other. In FIGURE 1 the state of affairs is represented schematically; in order to form the final products the reacting species are required to pass over the energy barrier by acquiring the activation energy E . From the point of view of its extra degree of translational freedom, which corresponds to its decomposition along the abscissa of the figure, the activated complex is regarded as being a free particle in a one dimensional box of length δ within which its partition function is $(2\pi m_M kT)^{1/2} \delta / h$. For the equilibrium between the reactants and the activated complex and intermediates we have the expression

$$K^{\ddagger} = \frac{a_M^{\ddagger} a_N a_O}{a_A a_B a_C} = \frac{c_M^{\ddagger} c_N c_O}{c_A c_B c_C} = \frac{\alpha_M^{\ddagger} \alpha_N \alpha_O}{\alpha_A \alpha_B \alpha_C} \quad (2)$$

where the a 's are the activities, the c 's the concentrations, and the α 's the activity coefficients with reference to the infinitely dilute gas

¹ Presented in part at the Conference on Kinetics in Solution held by the Section of Physics and Chemistry of the New York Academy of Sciences, February 24, 1939.

² Commonwealth Fund Fellow, Princeton University, 1938-40.

³ Stearn & Eyring, *Chem. Rev.* **24**: 253. 1939.

⁴ Eyring, *Jour Chem Phys* **3**: 107 1935

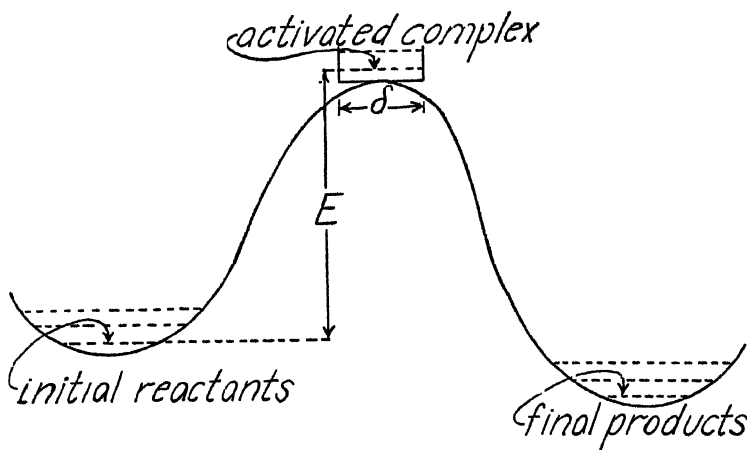


FIGURE 1.

where there are no interactions between the molecules. Otherwise, we may express the equilibrium constant in terms of the partition functions of the molecules:—

$$K^\ddagger = \frac{F_M^\ddagger F_N F_O \cdot}{F_A F_B F_C \cdot} \quad (3)$$

It is however convenient to separate out from the partition function F_M^\ddagger of the activated complex that part which refers to the degree of translational freedom corresponding to decomposition

$$F_M^\ddagger = F'_M \dagger \frac{(2\pi m_M \dagger kT)^{1/2} \delta}{h} \quad (4)$$

so that we may write the equilibrium constant as

$$K^\ddagger = \frac{F'_M \dagger F_N F_O \cdot}{F_A F_B F_C \cdot} \cdot \frac{(2\pi m_M \dagger kT)^{1/2} \delta}{h} \quad (5)$$

We may therefore write

$$K^\ddagger = K_0^\ddagger \frac{(2\pi m_M \dagger kT)^{1/2} \delta}{h} \quad (6)$$

where we have defined a new equilibrium constant K_0^\ddagger by

$$K_0^\ddagger \equiv \frac{F'_M \dagger F_N F_O \cdot \cdot \cdot}{F_A F_B F_C \cdot \cdot \cdot} \quad (7)$$

Combining (2) and (6) we obtain for the concentration of the activated complex the expression

$$c_M^\ddagger = K_0^\ddagger \frac{(2\pi m_M^\ddagger kT)^{1/2}}{h} \delta \frac{c_A c_B c_C \cdots \alpha_A \alpha_B \alpha_C \cdots}{c_N c_O \cdots \alpha_M^\ddagger \alpha_N \alpha_O \cdots} \quad (8)$$

The rate of the reaction is now given by the rate of decomposition of these complexes, which are assumed always to be in equilibrium with the initial reactants according to (2). The actual number of complexes per unit length of the potential energy box at any instant of time is of course given by the ratio of the concentration of the complexes (*i. e.* the number of complexes in the box) to the length of the box, *viz.* c_M^\ddagger/δ , and the reaction rate is given by this number multiplied by the average translational velocity in the direction of decomposition. If every complex formed eventually decomposed with the formation of the final products of the reaction this velocity can be shown to be given by the kinetic theory expression $(kT/2\pi m_M^\ddagger)^{1/2}$. Since however there is the possibility that some of the complexes will decompose in the wrong direction with the formation of the initial reactants, this expression is multiplied by a "transmission coefficient" κ which is equal to the probability of decomposition in the forward direction. The total expression for the rate therefore becomes

$$\begin{aligned} \text{Rate} &= K_0^\ddagger \frac{(2\pi m_M^\ddagger kT)^{1/2}}{h} \cdot \frac{c_A c_B c_C \cdots \alpha_A \alpha_B \alpha_C \cdots}{c_N c_O \cdots \alpha_M^\ddagger \alpha_N \alpha_O \cdots} \cdot \left(\frac{kT}{2\pi m_M^\ddagger} \right)^{1/2} \cdot \kappa \\ &= \kappa \frac{kT}{h} K_0^\ddagger \frac{c_A c_B c_C}{c_N c_O} \cdot \frac{\alpha_A \alpha_B \alpha_C \cdots}{\alpha_M^\ddagger \alpha_N \alpha_O \cdots} \end{aligned} \quad (9)$$

This is the actual rate of the reaction; if the specific rate k_1 is defined as the rate at which the reaction proceeds when the concentrations of $A, B, C \dots$ and of $N, O \dots$ are all unity it is given^{5, 6} by

$$k_1 = \kappa \frac{kT}{h} K_0^\ddagger \frac{\alpha_A \alpha_B \alpha_C \cdots}{\alpha_M^\ddagger \alpha_N \alpha_O \cdots} \quad (10)$$

The activity coefficients have been defined with reference to the dilute gas; in the dilute gas they are therefore unity and the rate in it is given by

$$k_{1(\text{gas})} = \kappa \frac{kT}{h} K_0^\ddagger \quad (11)$$

The rate of a reaction in solution is therefore equal to its rate in the

⁵ Wynne-Jones & Eyring, *Jour. Chem. Phys.* **3**: 493. 1935.

⁶ Stearn & Eyring, *Jour. Chem. Phys.* **5**: 113. 1937.

gas phase (if it here proceeds by exactly the same mechanism) multiplied by an activity coefficient term

$$\frac{\alpha_A \alpha_B \alpha_C \cdots}{\alpha_M^\dagger \alpha_N \alpha_O \cdots}.$$

We therefore have here a way of evaluating the rates of reactions in solution in terms of thermodynamic quantities, with a knowledge of which we have a complete theory. Owing, however, to the present lack of knowledge of the intermolecular forces operating in the liquid phase no complete treatment of a reaction in solution can be made. In many cases the best that one can as yet do is to make use of the empirical rules which have been put forward concerning the distribution of substances between the gaseous and liquid phases. In the present paper we attempt to interpret these rules in terms of the intermolecular forces and to show how reaction rates can be understood in the light of a theory of the thermodynamic behavior of solutions.

Before proceeding with a discussion of the influence of solvents on the activity coefficients α , a word may be said regarding the different activity coefficients which we shall have occasion to use. The activity coefficient α of a solute with reference to the dilute vapor is the ratio of the equilibrium concentrations in the vapor and in solution:—

$$\alpha = \frac{\text{concentration in vapor}}{\text{concentration in solution}},$$

the vapor being assumed to be ideal. In solution theory, however, it is frequently convenient to employ activity coefficients γ which refer to the pure liquids as having activity coefficients of unity, and which are therefore defined in general by

$$\gamma = \frac{\text{concentration in ideal solution}}{\text{concentration in actual solution}}.$$

For a given solute α and γ are clearly always proportional to one another, since

$$\alpha/\gamma = \frac{\text{concentration in vapor}}{\text{concentration in ideal solution}}.$$

The effects on α and γ of changing the solvent are therefore the same.

SHORT RANGE FORCES IN SOLUTION

In order to obtain some idea of the magnitudes of activity coefficients in solution it is necessary to consider some of the experimental facts regarding binary solutions, and some of the theories put forward

to explain them. Most of our information about solutions is obtained from the study of partial vapor pressure curves, which fall into three main classes:—

(1) In a limited number of cases straight lines are obtained, as is shown in FIGURE 2, curve *a*, Raoult's law being obeyed. Such be-

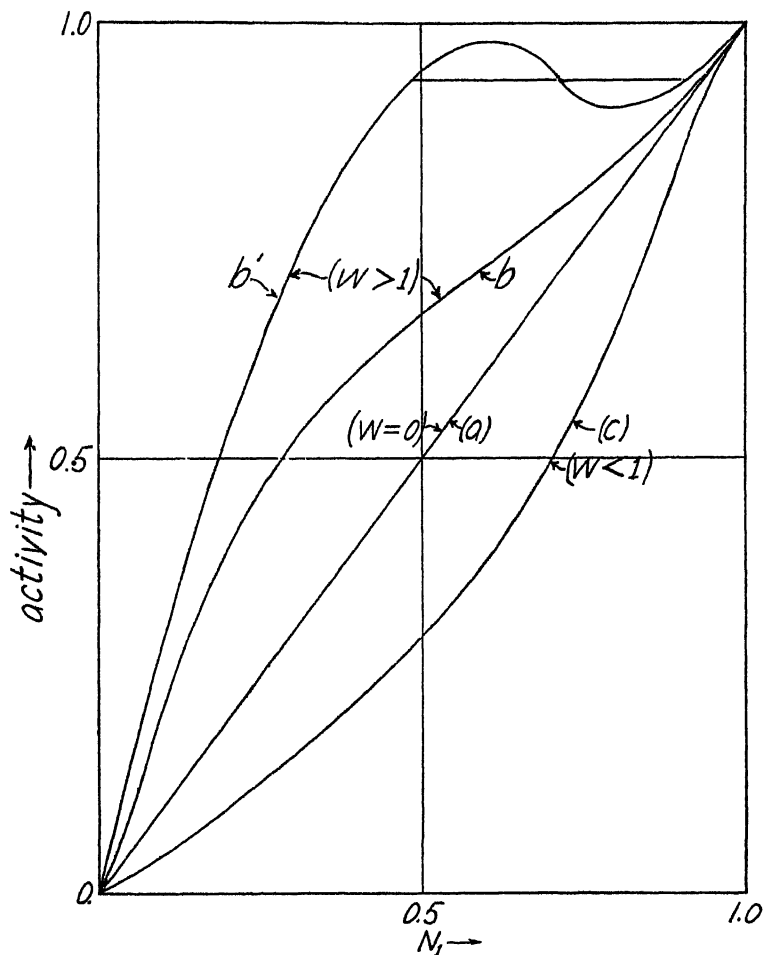


FIGURE 2. Activities, with reference to the pure liquids, plotted against mole fractions.

havior is probably only found when the constituents of the solution are of a similar chemical and physical nature, as for example with

¹ Young & Fortey, Jour. Chem. Soc. 83: 63. 1903.

² von Zawidski, Zeit. physikal. Chem. 35: 128. 1900.

chlorobenzene and bromobenzene,⁷ and ethylene bromide and propylene bromide.⁸ In these cases the activity coefficients γ_1 , γ_2 are unity.

(2) In a larger number of cases the curves lie above the ideal straight lines (FIGURE 2, curves *b* and *b'*), and the deviations from Raoult's law are said to be positive. Such curves are often found when the components of the solution are not very similar to one another, and when there is no evidence of any specific attraction between the different kinds of molecules. An interpretation of these curves will be given later.

(3) In other cases the deviations from Raoult's law are negative, the curves falling below the ideal straight line. Such behavior appears to be found under two sets of conditions:—

(a) When there is known to be chemical interaction between the different molecules.

(b) When one of the components of the solution is of high molecular weight. A number of such systems has been studied.⁹

It should be noted that, except in some cases in which very stable compounds are formed between the constituents of the solution, the limiting slope of the curve, as the mole fraction of each component approaches unity, is the correct Raoult's law slope. The explanation of this is as follows. From the experimental fact that partial vapor pressures are intensive and not extensive properties of solutions we infer that partial molal free energies are not functions of numbers of molecules, but of mole fractions only, and hence that total free energies, which give the partial molal free energies on partial differentiation, are linear in the numbers of molecules. From these facts alone¹⁰ follows the Gibbs-Duhem-Margules equation,

$$x_1 \frac{\partial \log p_1}{\partial x_1} = x_2 \frac{\partial \log p_2}{\partial x_2} \quad (12)$$

in which the x 's are mole fractions, and the p 's partial vapor pressures. By means of this equation it is very readily proved that if the initial slope (when the mole fraction is very small) of a vapor pressure versus mole fraction curve for one component is finite, the curve for the other component must have the correct Raoult's law slope in the same concentration region (*i. e.*, if $\partial p_1 / \partial x_1$ is finite as $x_1 \rightarrow 0$, the partial pressure curve of component 2 must approach the correct Raoult's law slope as x_2 approaches unity). This point is mentioned here since

⁹ See especially Brønsted & Colmant, *Zett. physikal. Chem.* A168:381. 1934. Boissonnas, *Helv. Chim. Acta* 20:768. 1934.

¹⁰ See *e. g.* Lewis & Randall, *Thermodynamics* 209. The equation can also be proved by statistical mechanics, as is done in ref. (14).

it shows that the only condition which must be imposed upon the partition function of a binary solution in order to obtain the correct limiting slope is that the total free energy shall be linear in the numbers of molecules; this is referred to again later.

Hildebrand¹¹ has defined as "regular" those solutions in which the distribution and orientation of the different molecular species are completely random, and in which therefore the molecules can rotate freely and there is no combination or association; that is, in which the entropy of mixing has the ideal value of

$$\Delta S = - R x_1 \log x_1 - R x_2 \log x_2. \quad (13)$$

The deviations from Raoult's law for these solutions he therefore regarded as being due to heat and not to entropy effects. Strictly speaking, however, such an assumption is not legitimate in a theory which is applied to solutions composed of liquids which are not completely miscible, and a modification of such theories of solution as those of Hildebrand and Wood,¹² and of Scatchard,¹³ in which random distribution of the molecules is assumed, is necessary. It will, however, be shown that the entropy deviations from ideality are always comparatively small, and that therefore the equations given by these theories are very useful from a practical point of view, since they will predict the general forms of vapor pressure curves even for mixtures the components of which have different molal volumes.

Treatments of solutions which take more accurate account of molecular "ordering" are those of Rushbrooke,¹⁴ and Kirkwood¹⁵ who have modified and improved an earlier treatment due to Guggenheim.¹⁶ The following assumptions are made:—

(1) That there is no volume change on mixing.

(2) That each molecule may be regarded as having a definite coordination number r which is the same for both components of the system (this is tantamount to assuming the molecules to have the same sizes and the same molal volumes). And

(3) That the total potential energy of the system is the sum of the contributions of neighboring pairs. If these potential energy contributions are $2w_1/r$, $2w_2/r$ and $2w_{12}/r$ for 1-1, 2-2 and 1-2 pairs respectively, and w is defined by the equation

¹¹ Hildebrand, Jour. Amer. Chem. Soc. 51: 66. 1929.

¹² Hildebrand & Wood, Jour. Chem. Phys. 1: 817. 1933

¹³ Scatchard, Chem. Rev. 8: 321. 1931.

¹⁴ Rushbrooke, Proc. Roy. Soc. A166: 296. 1938.

¹⁵ Kirkwood, Jour. Phys. Chem. 43: 94. 1939.

¹⁶ Guggenheim, Proc. Roy. Soc. A148: 304. 1935.

$$w \equiv 2w_{12} - w_1 - w_2 \quad (14)$$

it can be shown that the total potential energy is given by

$$W = N_1 w_1 + N_2 w_2 + Xw \quad (15)$$

where the N 's are the total numbers of molecules of the two types, and rX is the number of 1-2 pairs in the solution.

The partition function of a pure liquid can be written in the form

$$F_e = \left(\frac{2\pi mkT}{h^2} \right)^{\frac{3N}{2}} \int \dots \int e^{-W/kT} \prod_{K=0}^N (dv_K). \quad (16)$$

The Boltzmann factor in the above expression is integrated over the total volume of the liquid. In a similar manner the partition function of a solution is written as

$$F_s = J_1^{N_1} J_2^{N_2} \int \dots \int e^{-(N_1 w_1 + N_2 w_2 + Xw)/kT} (dv_1)^{N_1} (dv_2)^{N_2}, \quad (17)$$

where the J 's signify the free particle partition functions. The integration has now to be performed over the separate elements of volume corresponding to the two types of molecules. The above expression can be written as

$$F_s = J_1^{N_1} J_2^{N_2} e^{-(N_1 w_1 + N_2 w_2)/kT} \int \dots \int e^{-Xw/kT} (dv_1)^{N_1} (dv_2)^{N_2} \quad (18)$$

since the terms $N_1 w_1$ and $N_2 w_2$ are independent of the volume elements taken. In order to remove Xw from the integral it is necessary to define a parameter \bar{X} which is such that equation (18) is identically equal to

$$F_s = J_1^{N_1} J_2^{N_2} e^{-(N_1 w_1 + N_2 w_2 + \bar{X}w)/kT} \int \dots \int (dv_1)^{N_1} (dv_2)^{N_2} \quad (19)$$

The integral now has to be evaluated. Rushbrooke, following Fowler and Guggenheim, assumes that each molecule is confined within its own small free volume v_{f1} or v_{f2} and he therefore writes the integral as

$$v_{f1}^{N_1} v_{f2}^{N_2} \frac{(N_1 + N_2)!}{N_1! N_2!},$$

the factor $(N_1 + N_2)!/(N_1! N_2!)$ being the number of non-identical complexions of the system. According to Hirschfelder, Stevenson and Eyring^{17, 18} on the other hand, the free volumes are pooled among all of the molecules, so that the integral must be expressed as

$$\frac{[(N_1 + N_2)v_{f1}]^{N_1} [(N_1 + N_2)v_{f2}]^{N_2}}{N_1! N_2!}.$$

¹⁷ Eyring, Jour. Chem. Phys. 4: 283. 1936.

¹⁸ Hirschfelder, Stevenson & Eyring, Jour. Chem. Phys., 5: 896. 1937.

When the deviations from ideal behavior are calculated both of these expressions give identical results, since it is readily shown that according to either formulation the extent to which the Helmholtz free energy of mixing differs from the value it would have were the solution ideal is given by

$$\Delta A = \bar{\bar{X}}w. \quad (20)$$

It must however be pointed out that in the evaluation of the integral the free volumes appear in the form

$$v_{f1}^{N_1} v_{f2}^{N_2}$$

where v_{f1} and v_{f2} are the free volumes of the pure components 1 and 2; if another form were taken, for which the free volumes v_{f1} and v_{f2} were functions of the mole fractions, the energy would be found not to be linear in the numbers of molecules, and the partial molal free energies to be extensive properties of the system.

In order to obtain an expression for ΔA , which is equal to the Gibbs free energy ΔF since there is no volume change on mixing, an expression for $\bar{\bar{X}}$ has first to be found. Rushbrooke considers a group of $r+1$ molecules in the solution and applies the theory of order and disorder in a form first used by Bethe¹⁹ in the theoretical study of alloys. In this way he obtains the expression

$$\Delta F = \Delta A = \bar{\bar{X}}w = \frac{N_1 N_2}{N_1 + N_2} w \left\{ 1 - \frac{N_1 N_2}{(N_1 + N_2)^2} \left(\frac{rkT}{2w} e^{2w/rkT} - \frac{rkT}{2w} - 1 \right) \right\} \quad (21)$$

from which may be obtained the following expressions for ΔH , ΔS and the partial molal quantity $\Delta \bar{F}_1$:—

$$\Delta H = \Delta E = \frac{N_1 N_2}{N_1 + N_2} w \left\{ 1 - \frac{N_1 N_2}{(N_1 + N_2)^2} \left(e^{2w/rkT} - 1 \right) \right\} \quad (22)$$

$$\Delta S = -\frac{(N_1 N_2)^2}{(N_1 + N_2)^3} \left\{ \frac{rk}{2} \left(e^{2w/rkT} - 1 \right) - \frac{w}{T} e^{2w/rkT} \right\} \quad (23)$$

$$RT \log \gamma_1 = \Delta F_1 = w x_2^2 - x_1 x_2^2 (2x_2 - x_1) w \left\{ \frac{rkT}{2w} e^{2w/rkT} - \frac{rkT}{2w} - 1 \right\} \quad (24)$$

(γ_1 is the activity coefficient with reference to the pure liquid).

¹⁹ Bethe, *Proc. Roy. Soc. A150*: 552. 1935.

Kirkwood employs a different treatment but obtains finally an expression which corresponds with the completely expanded form of Rushbrooke's equation in the linear and quadratic terms in w . The conclusions based on the two treatments are therefore essentially the same. Kirkwood has calculated values of the energies and entropies under various conditions and has shown that the entropy terms due to ordering are always comparatively small, their contribution to the free energy term ΔF being in the neighborhood of critical conditions only of the order of 15%; in dilute solutions they are therefore often negligible. This clearly shows that in the majority of cases the treatments of Scatchard¹² and of Hildebrand and Wood,¹³ which are more general than those of Rushbrooke and Kirkwood in that they apply to mixtures of components of unequal volumes, are adequate. The formula of Hildebrand and Wood can be most generally expressed as

$$RT \log \gamma_1 = v_1 \left(\frac{x_2 v_2}{x_1 v_1 + x_2 v_2} \right)^2 \left(-\frac{2E_{12}}{\sqrt{v_1 v_2}} + \frac{E_{11}}{v_1} + \frac{E_{22}}{v_2} \right) \quad (25)$$

where the v 's are the molal volumes and the E 's the potential energy terms. In the special case of van der Waals forces, for which $E_{12} = (E_{11}E_{22})^{1/2}$ ²⁰ the equation becomes

$$RT \log \gamma_1 = v_1 \left(\frac{x_2 v_2}{x_1 v_1 + x_2 v_2} \right)^2 \left[\left(\frac{E_{11}}{v_1} \right)^{1/2} - \left(\frac{E_{22}}{v_2} \right)^{1/2} \right]^2 \quad (26)$$

It will be seen that equation (25) is identical with the linear term in w of Rushbrooke's equation when the volumes are equal. The higher terms in w in this latter equation express the contribution due to the ordering brought about by the forces in the solution.

All of these expressions predict the correct forms of the vapor pressure curves found experimentally; when $v_1 = v_2$ they give, for example, the useful empirical relation of Porter²¹ for the logarithms of the activity coefficients:—

$$\log \gamma_1 = \beta_1 x_2^2 \quad \text{and} \quad \log \gamma_2 = \beta_2 x_1^2 \quad (27)$$

The interpretation of the experimental results in terms of such theoretical expressions can be done very simply. If activities, calculated, for example, from Rushbrooke's equation (24), are plotted against mole fractions, it is found that Raoult's law is obeyed throughout the whole concentration range in the case that w is zero, whereas the deviations from the law are negative when w is negative, and positive

²⁰ London, *Zelt. physikal. Chem.* **B11**: 221. 1930.

²¹ Porter, *Trans. Faraday Soc.* **16**: 236. 1921.

when it is positive. In the last case when w is greater than the critical value given by $e^{-w/rkT} = 1 - 2/r$ the familiar critical conditions, and the associated separations into two layers are found. The physical interpretation of w is obtained from equation (14); its value tells us the extent to which the van der Waals attraction between molecules 1-2 differs from the average of the attractions between 1-1 and 2-2 molecules. Since the energies are positive in the case of repulsion a negative value of w corresponds to an attraction between the molecules of the different types which is greater than for an ideal solution, while a positive value means that this attraction is abnormally small. All of this agrees with the chemical evidence regarding the interactions between the different types of molecules.

With these theories it is possible to estimate the activity coefficients in solution of molecular species, including activated complexes which may usually be assumed to have properties intermediate between those of the reactants and the products of the reactions. The effect of solvents on reactions may therefore be predicted by introducing these coefficients into equation (10).

In all of the above theories the intermolecular field has been assumed, according to condition (3), to be composed of short range forces, but in many solutions we have also to recognize contributions from other types of force. In ionic solutions and solutions containing dipolar molecules there must be large long range electrostatic interactions. In these it is often reasonable to suppose that the electrostatic forces are the more important, since a reasonably accurate interpretation of the activity coefficients of ions in solutions and of the components of mixtures of such strongly dipolar substances as water, alcohols, amines, etc., can be made by considering only the electrostatic forces. It is, however, important to remember that the van der Waals forces are always present, although their effects may be masked, and we find it convenient to retain terms corresponding to them in all of the equations for reaction rates that we develop in this paper.

The above theories are not confined to solutions in which the forces are of the van der Waals type; the only restriction is that the forces shall be short range. The theories are therefore applicable to solutions in which chemical forces are operating; in these the forces are attractions, and w and the deviations from Raoult's law are negative. This is of great importance in the theory of reaction rates; for example the assumption of solvation, supported by the chemical evidence, is necessary to explain the influence of "polar" solvents on certain reactions.

We will consider first reactions in which the van der Waals forces are all-important and the dipole forces unimportant; secondly, reactions between ions; thirdly, reactions between neutral molecules where the electrostatic dipolar interactions are important; and finally reactions between ions and neutral molecules.

REACTIONS INVOLVING SHORT RANGE FORCES

Reactions involving short range forces may be divided into two classes according to whether or not they will take place homogeneously in the gas phase, and by the same mechanism as in solution. A few reactions of the first class have been studied accurately; they include the thermal decompositions of chlorine monoxide^{27, 23, 24} and nitrogen pentoxide,^{25, 26} the bimolecular decomposition of ozone, both uncatalysed,^{27, 28} and catalysed by chlorine,^{28, 29} the reaction between nitric oxide and chlorine,^{30, 31} the ortho-para hydrogen conversion in the presence of oxygen,³² and certain Diels-Alder reactions.³³ In certain solvents which have been defined as inert³⁴ these reactions proceed at the same rate as in the gas phase; in other solvents the rates differ, but rarely to any very great extent. Equal rates in solution and in the gas phase means that the ratio in equation (10) is unity. This situation arises from the fact that the activated complex resembles the reactants in properties to such an extent that the activity coefficients cancel one another out without themselves necessarily being nearly equal to unity; this may be expected particularly in unimolecular reactions where the differences between the normal and activated state are generally in the interiors of the molecules. Wassermann has suggested however that there is some such cancellation in certain bimolecular Diels-Alder reactions.

In certain solvents the same reactions occur with abnormal velocities. An example is the decomposition of nitrogen pentoxide which proceeds at an abnormally slow rate in nitric acid solution.²⁶ Here it is supposed that the nitrogen pentoxide is solvated by the

²² Hinshelwood & Pritchard, *Jour. Chem. Soc.* **123**: 2730 1923.

²³ Hinshelwood & Hughes, *Jour. Chem. Soc.* **125**: 1811 1924.

²⁴ Beaver & Steiger, *Zeit. physikal. Chem.* **B12**: 93. 1931.

²⁵ Daniels & Johnston, *Jour. Amer. Chem. Soc.* **43**: 53. 1921.

²⁶ Eyring & Daniels, *Jour. Amer. Chem. Soc.* **52**: 1472. 1929.

²⁷ Riesenfeld & Bohnholster, *Zeit. physikal. Chem.* **130**: 241. 1927.

²⁸ Bowen, Moelwyn-Hughes & Hinshelwood, *Proc. Roy. Soc. A* **134**: 211. 1931.

²⁹ Bodenstein, Patelt & Schumacher, *Zeit. physikal. Chem.* **B5**: 209. 1929.

³⁰ Trautz & Schueter, *Zeit. anorgan. Chem.* **136**: 1. 1924.

³¹ Trautz & Gerwig, *Zeit. anorgan. Chem.* **146**: 1. 1925.

³² Farkas & Sachsse, *Zeit. physikal. Chem.* **B23**: 1, 19. 1933.

³³ Wassermann, *Trans. Faraday Soc.* **34**: 128, 1938, and many other papers.

³⁴ See Moelwyn-Hughes, *Kinetics of Reactions in Solution* 68. 1933.

acid, and that there is a consequent lowering of its activity coefficient. The activated complex, however, is sufficiently different from the pentoxide not to form such a compound—or to form a weaker compound—with the result that its activity coefficient is not lowered to the same extent. The ratio $\alpha_{N_2O_5}/\alpha_{N_2O_5^\ddagger}$ is therefore less than unity and the reaction slow. This explanation is consistent with the energies of activation, an extra energy of 3,600 calories being needed in the nitric acid as compared with the inert solvents; this corresponds to the additional heat evolved in forming the compound between the normal nitrogen pentoxide and the solvent, as compared with that evolved in the case of the activated complex. A converse type of behavior to this is observed in the formation of quaternary ammonium salts in polar solvents, where it is the activated complex which is solvated; the result is an increased rate, and an energy of activation lowered by an amount related to the heats of formation of the solvating bonds.

Certain early attempts to correlate reaction rates with solubilities may now be considered in the light of the present theory. The activity coefficient γ of a solute is proportional to the reciprocal of its solubility L expressed as a mole fraction:—

$$\gamma \approx 1/L, \quad (28)$$

and since the activity coefficients α and γ are, for a given solute, directly proportional to one another, the rate of a reaction can be expressed in the form

$$k_1 = \text{const.} \times \frac{L_M^\ddagger L_N L_O \cdots}{L_A L_B L_C \cdots} \quad (29)$$

where "const." is a quantity not depending on the solvent. Dimroth³⁵ believed the correct equation to be

$$k_1 = \text{const.} \times \frac{1}{L_A L_B L_C \cdots} \quad (30)$$

and performed measurements on the conversion of the aci-form of 1-phenyl-5-oxytriazole carboxylic ester into its neutral form, finding a very good inverse proportionality between the specific rates and the solubilities of the reactants for a wide range of solvents for which the velocity constants varied by two powers of ten. As is to be expected, however, this relationship is by no means generally valid, and an opposite type of behavior has been found by von Halban,^{36, 37, 38} who employed the velocity equation:—

³⁵ Dimroth, *Liebig's Annalen* **377**: 127. 1910.

³⁶ von Halban, *Zeit. physikal. Chem.* **67**: 129. 1909.

³⁷ von Halban & Kirsch, *Zeit. physikal. Chem.* **82**: 325. 1913.

³⁸ von Halban, *Zeit. physikal. Chem.* **84**: 129. 1913.

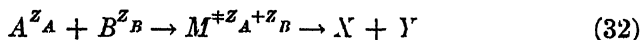
$$k_1 = \text{const.} \times L_X L_A L_Z \quad \dots \quad (31)$$

and found for the decompositions of substituted sulphonium salts, quaternary ammonium salts, and xanthogenic acid a direct proportionality between the specific rates and the solubilities of the final products. It is clear that these findings of Dimroth and von Halban are to a large extent fortuitous. It must be supposed that in Dimroth's reaction the variation in solubility of the activated complex was small, and that in those of von Halban the products resembled the activated complexes from the point of view of their behavior in solution, and that the variations in solubility of the reactants were small. However, the fact that when relationships of this sort are found the rates vary either inversely with the solubilities of the reactants, or directly with the solubilities of the products may be regarded as significant and as being general support for the theory.

REACTIONS INVOLVING LONG RANGE FORCES

Reactions between Ions

Reactions between ions can be treated in a more quantitative manner, since activity coefficients of ions in solution have been studied theoretically and experimentally in much more detail. For a bimolecular ionic reaction of the type



the logarithm of the specific reaction rate is given as before by the expression

$$\log k_1 = \log \left(\kappa \frac{kT}{h} K_0^+ \right) + \log \frac{\alpha_A \alpha_B}{\alpha_{M^*}} \quad (33)$$

It is here convenient to separate the activity coefficients α into the two coefficients β and f , of which β is the activity coefficient of the infinitely dilute solution with reference to the infinitely dilute gas, and f is that of the solution which is under consideration with reference to the infinitely dilute solution. We thus have the relationship

$$\alpha = \beta f \quad (34)$$

or

$$\log \alpha = \log \beta + \log f \quad (35)$$

In order to evaluate β we need to know the free energy of transfer of an ion from a vacuum to the medium of dielectric constant D . We suppose that the process takes place as follows. The ion is first dis-

charged in a vacuum ($D = 1$) when its free energy increase (in fact a decrease, the sign being negative) is given according to Born³⁹ by

$$-\frac{Z^2\epsilon^2}{2r}$$

where r is the radius of the conducting sphere upon which the charge may be supposed to be located. The uncharged ion is then transferred to the infinitely dilute solution, when it experiences a free energy increase Φ' due to the fact that there are van der Waals and possibly other forces between it and its neighbors in the solution; if necessary Φ' can be expressed in terms of the intermolecular forces by use of the theories of solutions and of pure liquids. Thirdly, the molecule is recharged in the solution, when its free energy increase is given by

$$\frac{Z^2\epsilon^2}{2rD}$$

During this process of recharging, however, the molecules around the ion will become polarized by the charge, and will therefore re-orient themselves with respect to it. For actual molecules this electrostriction is only partly accounted for by the dielectric constant term in the Born expression; in order to take account of the remainder we introduce a second non-electrostatic term Φ'' . The total increase in free energy is therefore

$$\Delta F = -\frac{Z_A^2\epsilon^2}{2r} + \frac{Z_A^2\epsilon^2}{2rD} + \Phi' + \Phi''. \quad (36)$$

We thus have for the activity coefficient β_A the expression

$$\log \beta_A = -\frac{Z_A^2\epsilon^2}{2kTr_A} \left(\frac{1}{D} - 1 \right) + \Phi_A'/kT + \Phi_A''/kT \quad (37)$$

and similar expressions for β_B and β_M . Hence

$$\log \frac{\beta_A \beta_B}{\beta_M^2} = -\frac{\epsilon^2}{2kT} \left(\frac{1}{D} - 1 \right) \left(\frac{Z_A^2}{r_A} + \frac{Z_B^2}{r_B} - \frac{(Z_A + Z_B)^2}{r_M^2} \right) + \frac{\Phi_A' + \Phi_A'' + \Phi_B' + \Phi_B'' - \Phi_M^{2'} - \Phi_M^{2''}}{kT}. \quad (38)$$

The activity coefficients f refer the solutions of ionic strength Γ to the infinitely dilute solutions, and are therefore given by the Debye-Hückel expression⁴⁰ with the addition of the non-electrostatic term Φ_i''' :—

³⁹ Born, *Zeit. Physik.* **1**: 45, 1920.

⁴⁰ Debye & Hückel, *Physikal. Zeit.* **24**: 185, 1923.

$$\log f_i = - \frac{Z_i^2 e^2}{2DkT} - \frac{K}{1+a_i K} + \Phi_i'''/kT. \quad (39)$$

K is given by

$$K^2 = \frac{8\pi e^2}{DkT} \Gamma, \quad \left(\Gamma = \frac{1}{2} \sum_i n_i Z_i^2 \right) \quad (40)$$

In the derivation of the Debye-Huckel equation the ions are regarded as being hard spheres, and a_i is the average distance of closest approach of the particular ion i in question to the other ions in the solution; it is clearly the distance of closest approach of the various ions weighted by the frequency of their collisions with the ion i . The non-electrostatic effects are therefore treated as if they were entirely repulsions beginning suddenly at this distance. The Φ''' terms modify this so as to take into account the van der Waals attractions which may be of considerable importance.^{40a} It is clear that a_i will have different values for different ions which are in solution with the ion i . Combining the expressions for the activity coefficients of A , B and M^{\pm} we have

$$\log \frac{f_A f_B}{f_{M^{\pm}}} = - \frac{e^2}{2DkT} \left(\frac{Z_A^2 K}{1+a_A K} + \frac{Z_B^2 K}{1+a_B K} - \frac{(Z_A+Z_B)^2 K}{1+a_{M^{\pm}} K} \right) + \frac{\Phi_A''' + \Phi_B''' - \Phi_{M^{\pm}}'''}{kT}. \quad (41)$$

Since it is the case that the a 's are not of great importance at low ionic strengths, and that their effects become vanishingly small as the limit of zero ionic strength is approached, it is convenient here to follow the usual practice and make use of a single a term, so effecting a considerable simplification in the form of equation (41). This a has not a simple physical significance in that it is a mean of three terms which are themselves mean values. We write, as an approximation,

$$a_A = a_B = a_{M^{\pm}} = a \quad (42)$$

whence

$$\log \frac{f_A f_B}{f_{M^{\pm}}} = \frac{Z_A Z_B e^2}{DkT} \frac{K}{1+aK} + \frac{\Phi_A''' + \Phi_B''' - \Phi_{M^{\pm}}'''}{kT}. \quad (43)$$

The complete expression for the rate of the reaction therefore becomes, if we write $\Phi_A = \Phi_A' + \Phi_A'' + \Phi_A'''$ etc.,

^{40a} A quantitative theory of ionic solutions which takes into account the short range van der Waals forces has been developed by Guggenheim, *Phil. Mag.* **19**: 588. 1935. Cp. Eley & Evans, *Trans. Faraday Soc.* **34**: 1093. 1938.

$$\log k_1 = \log \left(\kappa \frac{kT}{h} K_0^+ \right) + \frac{\epsilon^2}{2kT} \left(\frac{1}{D} - 1 \right) \\ \left(\frac{Z_A^2}{r_A} + \frac{Z_B^2}{r_B} - \frac{(Z_A + Z_B)^2}{r_M^{\ddagger}} \right) + \frac{Z_A Z_B}{DkT} \frac{K}{1 + aK} + \frac{\Phi_A + \Phi_B - \Phi_M^{\ddagger}}{kT} \quad (44)$$

in which the approximation of equation (42) has been retained.

This formula may be contrasted with that obtained by Scatchard⁴¹ who based his treatment on an earlier theory of Christiansen.⁴² If k_0 is the rate of the reaction in the gas phase when the ionic strength is zero and the dielectric constant unity, Scatchard's formula can be written as

$$\log k_1 = \log k_0 - \frac{\epsilon^2}{kT} \left(\frac{1}{D} - 1 \right) \frac{Z_A Z_B}{r_A + r_B} + \frac{Z_A Z_B}{DkT} \frac{K}{1 + aK} \quad (45)$$

It is seen that the second term of this, which should be the counterpart of the Born term in (44), has a different form from the latter; no simple approximation in either will make the two terms the same. As Scatchard agrees,^{42a} the difference is in models rather than in the fundamental theory. In his treatment the activated complex is assumed to be a double sphere, the spheres bearing the charges of the original reactants; in ours the activated complex is taken as spherical both as to shape and charge distribution.

An application of the Christiansen-Scatchard formula to the reaction between bromoacetate and thiosulphate ions has recently been made by LaMer,⁴³ who evaluates $r_A + r_B$ from the slope of the line obtained by plotting the logarithm of the rate extrapolated down to zero ionic strength against the reciprocal of the dielectric constant. This curve is shown in FIGURE 3, the data being taken from the work of LaMer and Kamner,⁴⁴ Fessenden,⁴⁵ Tomlinson,⁴⁶ and of Straup-Cope and Cohn,⁴⁷ and of Eagle and Warner,⁴⁸ all of whom used the sodium salts of the anions. The points are seen to lie on a good straight line, the slope of which corresponds to a value of $r_A + r_B$ in (45) equal to 5.1 Å. From the way in which the rate of the reaction varies with the ionic strength at constant dielectric, a has been found to be equal to 6.4 Å. As already mentioned a is only a mean value which varies according to

⁴¹ Scatchard, *Jour. Am. Chem. Soc.* **52**: 52. 1930. *Chem. Rev.* **10**: 229. 1932.

⁴² Christiansen, *Zeit. physikal. Chem.* **113**: 35. 1924.

^{42a} Scatchard, *Jour. Chem. Phys.* **7**: 657. 1939.

⁴³ LaMer, *Jour. Franklin Institute* **225**: 709. 1938.

⁴⁴ LaMer & Kamner, *Jour. Am. Chem. Soc.* **57**: 2660. 1935.

⁴⁵ LaMer & Fessenden, *Jour. Am. Chem. Soc.* **54**: 2351. 1932.

⁴⁶ Tomlinson, Ph.D. Thesis, Columbia University. 1939.

⁴⁷ Straup-Cope & Cohn, *Jour. Am. Chem. Soc.* **57**: 1794. 1935

⁴⁸ Eagle & Warner, *Jour. Am. Chem. Soc.* **58**: 2335. 1936.

what other ions are present in the solution, and therefore close agreement between the value of a and $r_A + r_B$ is not to be expected. The value of $r_A + r_B$ which is found on this theory is of course reasonable.

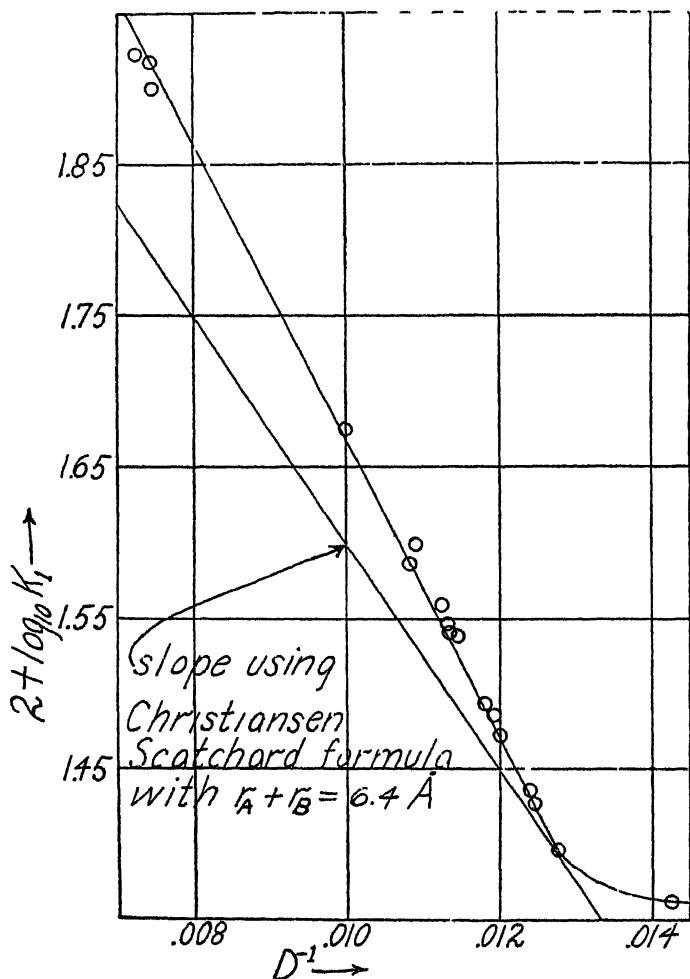


FIGURE 3. The logarithm of the specific rate extrapolated to zero ionic strength plotted against the reciprocal of the dielectric constant, for the bromoacetate-thiosulphate reaction, $\text{BrOH}_2\text{COO}^- + \text{S}_2\text{O}_3^{2-} =$. The slope of the line through the points corresponds to the following radii $r_{\text{bromoacetate}} = 3.3 \text{ \AA}$; $r_{\text{thiosulphate}} = 1.7 \text{ \AA}$; $r_{\text{activated complex}} = 5.0 \text{ \AA}$. $T = 25^\circ \text{C}$.

It is unfortunate that values for the ionic radii are not available, since as yet a precise application of (44) and (45) is impossible; however, when reasonable values of the ionic radii of the reacting ions and the activated

complex for this reaction are taken ($r_{\text{bromoacetate}} = 3.3 \text{ \AA}$; $r_{\text{thiosulphate}} = 1.7 \text{ \AA}$; $r_{\text{complex}} = 5.0 \text{ \AA}$) the slope of $\log k_{\Gamma=0}$ against $1/D$ can be explained exactly on our model. It is thus clear that neither (14) nor (45) is inconsistent with the results, and that further work is required to establish which is the better model of the two.

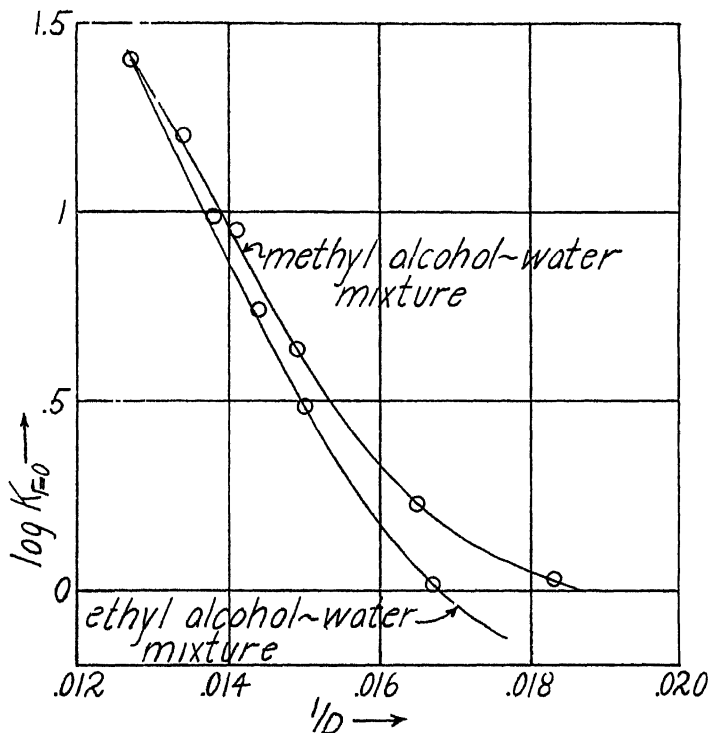


Figure 1 Reaction between hydroxyl ions and bromphenol blue, $(\text{BrPhB}) + \text{OH}^-$. The logarithm of the specific rate extrapolated to zero ionic strength plotted against the reciprocal of the dielectric constant. Temperature = 25°C

In FIGURE 3 considerable changes of slope are found at values of $1/D$ above .013; the effect is certainly real since determinations giving other points at lower dielectric constants have confirmed it.^{44, 46} The effect amounts to a diminished influence of dielectric constant on the rate of the reaction at low dielectric constants. The explanation is most probably that in a mixed solvent containing water there will be a preferential adsorption of water on the ions with the result that when solvents of lower dielectric constant are added their effect will

not be as great as if the solvent molecules were randomly distributed.⁴³ Another example of the phenomenon has recently been provided by

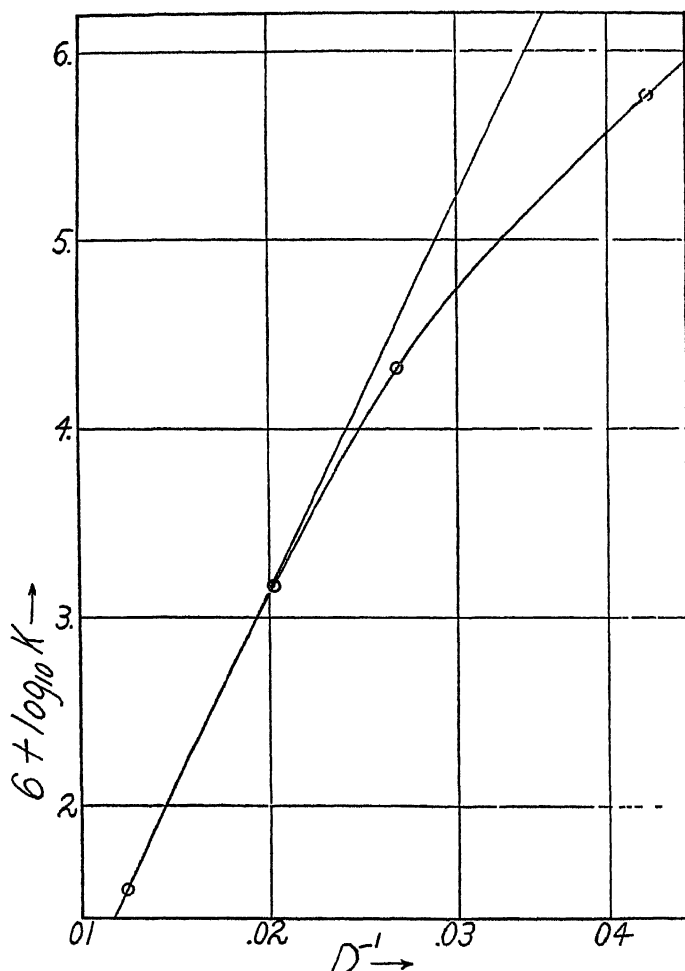


FIGURE 5. Hydrolysis by hydroxyl ions of the trimethylsulphonium cation; $\text{Me}_3\text{S}^+ | \text{OH}^-$. Plot of $\log k$ against $1/D$. $T = 20^\circ \text{C}$.

Amis and LaMer⁴⁹ for the reaction between bromphenol blue and hydroxyl ions, some of the results for which are shown plotted in

^{43a} Similar deviations at low dielectric constants, probably due to the same cause, are found in other works (e. g., Minnick & Kilpatrick, *Jour. Phys. Chem.* **43**: 259, 1939, acid-base equilibria; Harned & Embree, *Jour. Am. Chem. Soc.* **57**: 1669, 1935, Harned & Kazanjia, *Jour. Am. Chem. Soc.* **58**: 1812, 1936, dissociation constant of acetic acid).

⁴⁹ Amis & LaMer, *Jour. Am. Chem. Soc.* **61**: 905, 1939.

FIGURE 4. These results are also interesting in that they show the effect of the non-electrostatic forces, the slopes being different for ethyl alcohol—water and methyl alcohol—water mixtures. Other results which may also be interpreted in terms of preferential adsorption of water are those for the hydroxyl ion hydrolysis of the trimethyl sulphonium cation,⁵⁰ plotted in FIGURE 5. Here, owing to the fact that the reacting ions have opposite charges the effect of changing the dielectric constant is the opposite to what it is in the above examples (see later), but again the rates at low dielectric constants are more like those at high dielectric constants than would be found if the solvent molecules were distributed at random.

Reactions between Dipolar Substances

When we come to deal with reactions in which the reactants are uncharged it is certain that the van der Waals forces cannot be neglected except as a very rough approximation. The evidence, however, is that in the cases of solutions containing water, alcohols, amines, etc., the dipolar forces are of somewhat greater importance than the non-electrostatic ones. It is therefore natural to attempt to treat certain reactions involving fairly strong dipoles in terms of the electrostatic dipolar forces. The type of treatment to be described here has been applied in some detail, but in a rather different manner, by Stearn and Eyring.⁵¹

Kirkwood⁵² has given for the free energy of transfer of a strong dipole of moment μ from a vacuum to a medium of dielectric constant D an expression which, when the charge distribution within the molecule is symmetrical, becomes

$$\Delta F = kT \log \beta = - \frac{\mu^2}{a^3} \left[\frac{D-1}{2D+1} \right] \quad (46)$$

where a is the molecular radius. Correcting this as was done above by the addition of a non-electrostatic term Φ we have

$$\Delta F = kT \log \beta = - \frac{\mu^2}{a^3} \left[\frac{D-1}{2D+1} \right] + \Phi. \quad (47)$$

Other less accurate expressions than that of Kirkwood had previously been given by Martin,⁵³ and Bell.⁵⁴ Bell⁵⁵ has also shown that ex-

⁵⁰ Gleave, Hughes & Ingold, *Jour. Chem. Soc.* 236 (1935).

⁵¹ Stearn & Eyring, *Jour. Chem. Phys.* 5: 113. 1937.

⁵² Kirkwood, *Jour. Chem. Phys.* 2: 351. 1934.

⁵³ Martin, *Phil. Mag.* 8: 547. 1929.

⁵⁴ Bell, *Trans. Faraday Soc.* 27: 797. 1931.

⁵⁵ Bell, *Trans. Faraday Soc.* 31: 1557. 1935.

pression (46) cannot be expected to apply when the dielectric constant of the medium is low, and this has been found to be the case; but the expression is of great value in dealing with fairly concentrated solutions of polar molecules, in which it can be assumed that the electrostatic forces are of primary importance. Very good agreement between the theory and experiment has been found, particularly by

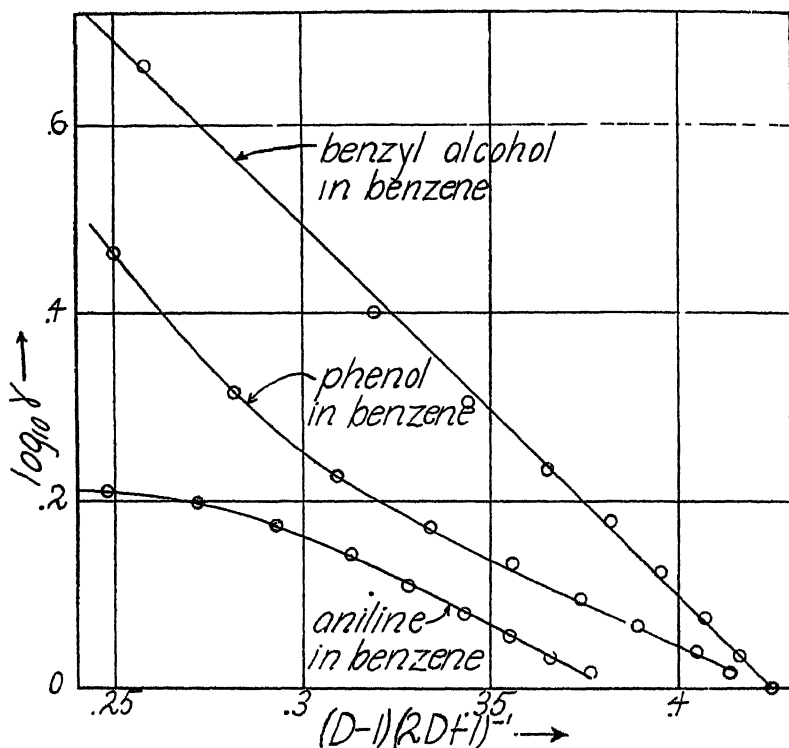


FIGURE 6 Plot of the logarithm of the activity coefficient (γ) against the function $(D - 1)/(2D + 1)$ for mixtures of some dipolar molecules with benzene. $T = 70^\circ \text{C}$.

Martin and his collaborators,⁵⁶⁻⁶⁰ who have obtained satisfactory straight lines of reasonable slopes on plotting the logarithms of the activity coefficients against the function $(D - 1)/(2D + 1)$, deviations from linearity only being found at low dielectric constants. Some typical curves are shown in FIGURE 6.

⁵⁶ Martin & Collie, Jour. Chem. Soc. 2665 (1932).

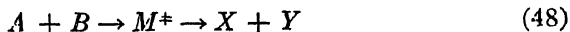
⁵⁷ Martin, Trans. Faraday Soc. 30: 759. 1934.

⁵⁸ Martin & George, Jour. Chem. Soc. 1413 (1933)

⁵⁹ Martin, Trans. Faraday Soc. 33: 191. 1937.

⁶⁰ Martin & Brown, Trans. Faraday Soc. 34: 742. 1938.

Applying equation (47) to the bimolecular reaction



we obtain the expression

$$\log k_1 = \log \left(\kappa \frac{kT}{h} K_0^{\ddagger} \right) - \frac{1}{kT} \frac{D-1}{2D+1} \left[\frac{\mu_A^2}{a_A^3} + \frac{\mu_B^2}{a_B^3} - \frac{\mu_{M^{\ddagger}}^2}{a_{M^{\ddagger}}^3} \right] + \frac{\Phi_A + \Phi_B - \Phi_{M^{\ddagger}}}{kT} \quad (49)$$

where D is the dielectric constant of the final solution formed. Provided the non-electrostatic terms are small enough to be neglected as

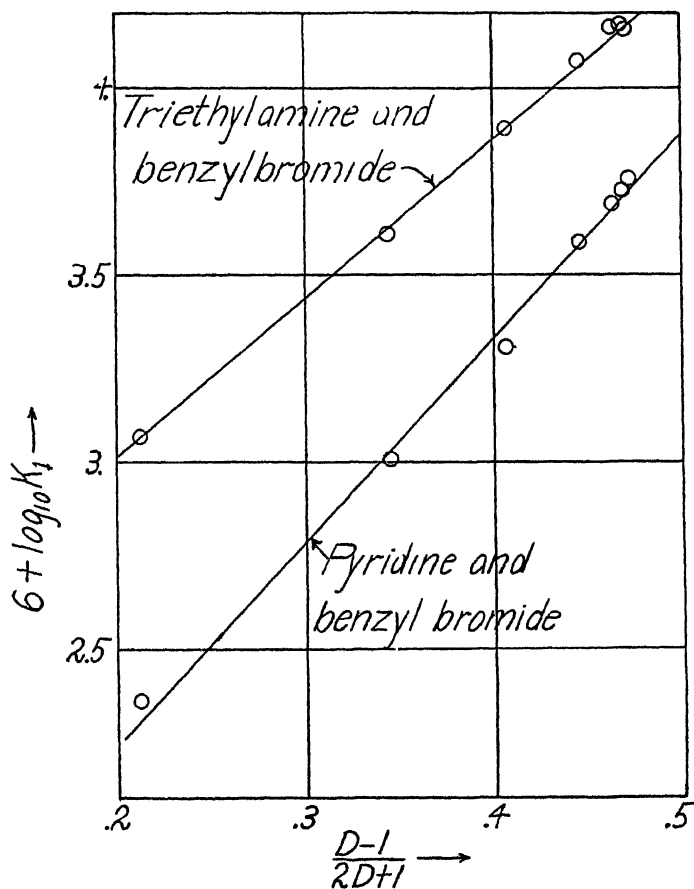


FIGURE 7. The logarithm of the specific rate plotted against $(D-1)/(2D+1)$ for quaternary ammonium salt formations in alcohol-benzene mixtures. $T = 29^\circ \text{C}$.

an approximation we expect to obtain straight lines on plotting the logarithms of the specific rates against $(D - 1)/(2D + 1)$; or, since the dielectric constants of dilute solutions do not differ greatly from those of the pure solvents, against the corresponding function in which the more available dielectric constants of the pure solvents

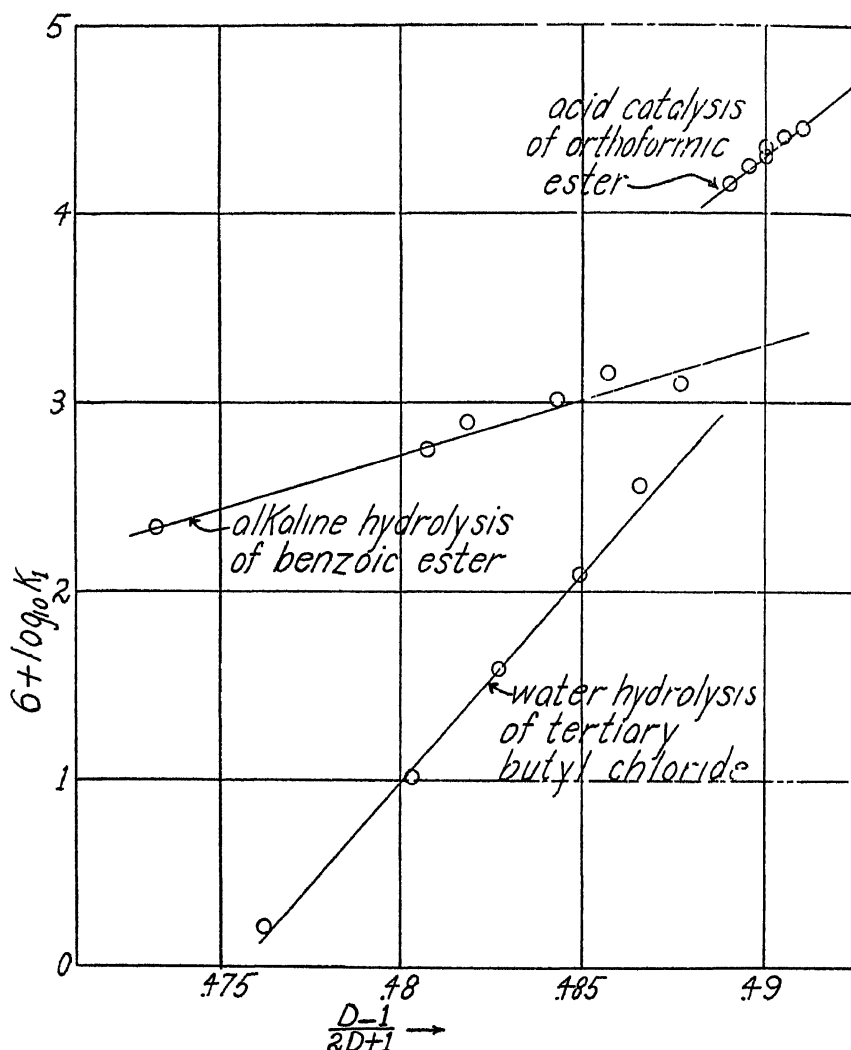


FIGURE 8. The logarithm of the specific rate plotted against $(D - 1)/(2D + 1)$ for the acid hydrolysis of ethyl orthoformate ($\text{H}_2\text{O} + \text{HC}(\text{OEt})_3$), the alkaline hydrolysis of ethyl benzoate ($\text{H}_2\text{O} + \text{PhCOOEt}$), and the water hydrolysis of tertiary butyl chloride ($\text{H}_2\text{O} + (\text{CH}_3)_3\text{CCl}$).

are used. This has been done in FIGURE 7 for two quaternary ammonium salt formations^{60a} in alcohol-benzene mixtures,⁶¹ and in FIGURE 8 for the water hydrolysis of tertiary butyl chloride,⁶² for the acid-catalysed hydrolysis of orthoformic ester,⁶³ and for the alkaline hydrolysis of ethyl benzoate,⁶⁴ the last three in alcohol-water mixtures; it is shown later that from the point of view of solvent influence an ester hydrolysis reaction should be regarded as a reaction between two dipoles, the ester and the water molecule. In these five cases the agreement with the theory is as good as can be expected. On the other hand when nitrobenzene is one of the components of the solvent considerable deviations are found, as is shown in FIGURE 9, in which the same reactions as are plotted in FIGURE 7 are plotted for benzene-nitrobenzene mixtures.⁶⁴ It is seen that in solutions rich in nitrobenzene the reaction proceeds much faster than the theory predicts. Moreover a careful examination of the data concerning the formation of quaternary ammonium salts shows that such specific solvent effects are very common. Grimm, Ruf and Wolff,⁶⁵ for example, have studied the reaction between triethylamine and ethyl iodide in a variety of different solvents, and their results show that the rates and the dielectric constants are not even in the same order as one another, although, as was pointed out by Menshutkin,⁶⁶ there is a rough correlation between the two, in that the reaction does tend to be accelerated by solvents of high dielectric constant. The explanation of the specific effects can be formulated in terms of the non-electrostatic terms Φ . Probably the most important effect contributing to these terms is the solvating power of the solvents. Thus, examination of the solubilities⁶⁷ of amines, iodides and quaternary ammonium salts in benzene and nitrobenzene, and of the conductivities⁶⁸ of the resulting solutions, has shown that in nitrobenzene the quaternary salts are soluble and ionised, and therefore probably solvated,^{68a} whereas in benzene they are only slightly soluble. The solutions of the amines and

^{60a} The numerous results of Fairclough and Hinshelwood on the pyridine-methyl iodide reaction (Refs. 64 and 88) show considerable deviations from linearity; this is attributed to the fact that in methyl iodide the dipole is imbedded in the molecule with the result that the dipolar effects will be subsidiary.

⁶¹ Muchin, Ginsberg & Moissejewa, *Chem. Zentr.* 2: 2376. 1926.

⁶² Hughes, *Jour. Chem. Soc.* 255. 1935.

⁶³ Harned & Samaras, *Jour. Am. Chem. Soc.* 54: 9. 1932.

⁶⁴ Fairclough & Hinshelwood, *Jour. Chem. Soc.* 538 (1937).

⁶⁵ Grimm, Ruf & Wolff, *Zeit. physikal. Chem.* B13: 301. 1931.

⁶⁶ Menshutkin, *Zeit. physikal. Chem.* 6: 41. 1890.

⁶⁷ E. g. Bjerrum & Jozefowicz, *Zeit. physikal. Chem.* A159: 194. 1932.

⁶⁸ E. g. Walden, *Bull. Acad. Sci. Petersburg* 7: 427. 1913.

^{68a} The term "solvated" is here used in its most general sense to refer to any form of association between solute and solvent which is accompanied by heat evolution.

iodides however are normal, in that their deviations from ideality are probably not large. Stearn and Eyring⁵¹ have shown that whereas in the formation of quaternary ammonium salts the activated complex is not ionic, it has a considerable dipole moment, and therefore can be regarded as approaching the salt in general properties. Hence we may suppose that the high velocity of the reactions in nitrobenzene is due

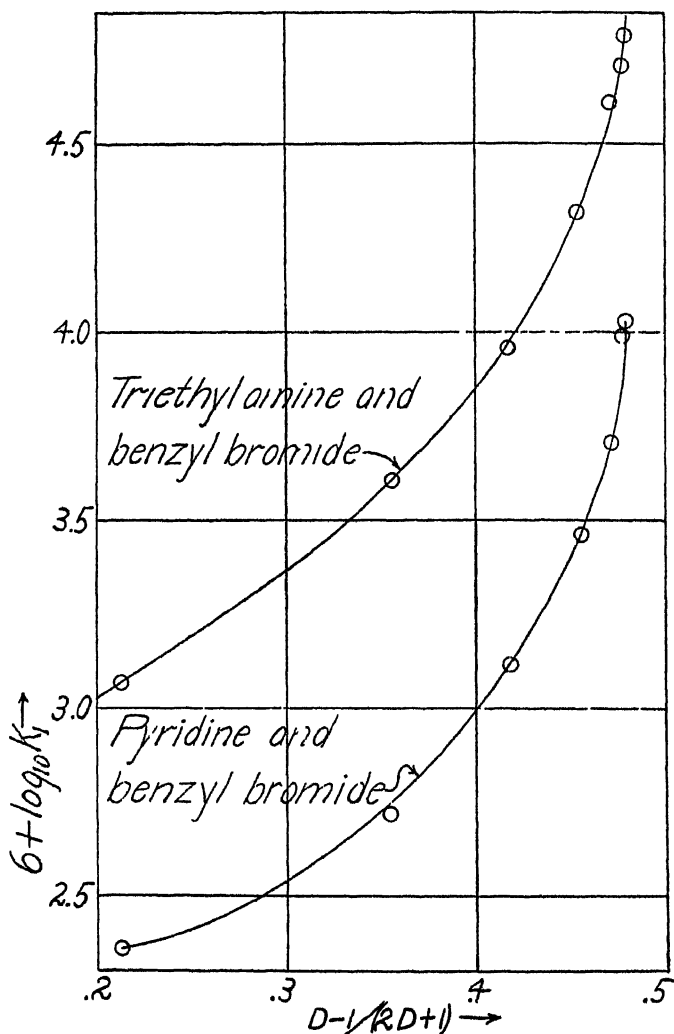


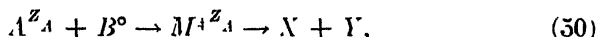
FIGURE 9. The logarithm of the specific rate plotted against $(D - 1)/(2D + 1)$ for quaternary ammonium salt formations in benzene-nitrobenzene mixtures. $T = 20^\circ \text{C}$.

to the solvation of the activated complex, on account of which its activity coefficient is very low. In terms of the Φ functions we may say that even in nitrobenzene Φ_A and Φ_B are fairly normal, but that owing to the solvation the term Φ_M^\ddagger is very small. Benzene may be considered to have the converse effect to nitrobenzene, for since the solubility in it of the activated complex is low (this we infer from the fact that the product of the reaction is only slightly soluble) α_M^\ddagger and Φ_M^\ddagger will be large and the reaction slow. The fact that the Menschutkin reaction will not proceed in the gas phase, however, shows that even in benzene the solvent must effect some sort of stabilization of the partially ionised complex.

It is clear that before these reactions can be treated more quantitatively many more activity coefficients need to be determined, such data being particularly meager at present.

Reactions between Ions and Neutral Molecules

We consider reactions of the general type



where $z_A \epsilon$ is the charge on A and M^\ddagger . The activity coefficients of the ions A and M^\ddagger are given by the expressions

$$\log \alpha_A = \frac{Z_A^2 \epsilon^2}{2r_A kT} \left(\frac{1}{D} - 1 \right) - \frac{Z_A^2 \epsilon^2}{2DkT} \frac{K}{1 + a_A K} + b_A \Gamma + \frac{\Phi_A}{kT} \quad (51)$$

$$\log \alpha_M^\ddagger = \frac{Z_A^2 \epsilon^2}{2r_A kT} \left(\frac{1}{D} - 1 \right) - \frac{Z_A^2 \epsilon^2}{2DkT} \frac{K}{1 + a_M^\ddagger K} + b_M^\ddagger \Gamma + \frac{\Phi_M^\ddagger}{kT} \quad (52)$$

in which we have now included the semi-empirical $b\Gamma$ terms of Huckel⁶⁹ in addition to the non-electrostatic terms Φ . To obtain an expression for α_B we employ the expressions for the free energy of transfer of a neutral molecule from the vapor to a medium of dielectric constant D_0 (which is that of the solution to which B itself has been added, but in which the ions are not yet present), and then for the free energy increase brought about by the addition of the ions. For the first of these we use the Kirkwood formula modified by the introduction of the non-electrostatic terms (equation (47)), for the second the approximate expression given by Debye and McAulay⁷⁰ which relates the free energy of the process to the extent to which the dielectric constant of the solvent is altered by the addition of the non-electrolyte:—

⁶⁹ Huckel, *Physikal. Zeit.* **26**: 93. 1925.

⁷⁰ Debye & McAulay, *Physikal. Zeit.* **26**: 22. 1925

$$\Delta F = kT \log f = \frac{\bar{\alpha}\epsilon^2}{2D} \sum_i \frac{Z_i^2 n_i}{r_i} \quad (53)$$

where n_i is the number of ions of type i and radius r_i per cc.; $\bar{\alpha}$ is defined by

$$D' = D_0' (1 - \bar{\alpha} n_B) \quad (54)$$

where D_0' is the dielectric constant of the pure solvent, and D' that of a solution containing n_B molecules of the non-electrolyte B per cc. The activity coefficient can therefore be expressed as

$$\log \alpha_B = -\frac{1}{kT} \left[\frac{D_0 - 1}{2D_0 + 1} \right] \frac{\mu_B^2}{a_B^3} + \frac{\bar{\alpha}\epsilon^2}{2DkT} \sum_i \frac{Z_i^2 n_i}{r_i} + \frac{\Phi_B}{kT} \quad (55)$$

and we have finally for the rate of the reaction the expression

$$\begin{aligned} \log k_1 = \log \left(\kappa \frac{kT}{h} K_0^\ddagger \right) + \frac{Z_A^2 \epsilon^2}{2kT} \left(\frac{1}{D} - 1 \right) \left(\frac{1}{r_A} - \frac{1}{r_{M^\ddagger}} \right) - \frac{Z_A^2 \epsilon^2}{2DkT} \\ \left(\frac{K}{1 + a_A K} - \frac{K}{1 + a_{M^\ddagger} K} \right) - \frac{1}{kT} \left[\frac{D_0 - 1}{2D_0 + 1} \right] \frac{\mu_B^2}{a_B^3} + \left(b_A - b_{M^\ddagger} + \frac{\bar{\alpha}\epsilon^2}{DrkT} \right) \Gamma \\ + \frac{\Phi_A + \Phi_B - \Phi_{M^\ddagger}}{kT} \quad (56) \end{aligned}$$

where r is an average value of the r_i 's. In the limit of very small ionic strengths the mean radii a_A and a_{M^\ddagger} can be regarded as equal, since their effects as $\Gamma \rightarrow 0$ become vanishingly small, and we therefore have for the rate

$$\begin{aligned} \log k_1 = \log \left(\kappa \frac{kT}{h} K_0^\ddagger \right) + \frac{Z_A^2 \epsilon^2}{2kT} \left(\frac{1}{D} - 1 \right) \left(\frac{1}{r_A} - \frac{1}{r_{M^\ddagger}} \right) - \frac{1}{kT} \\ \left[\frac{D_0 - 1}{2D_0 + 1} \right] \frac{\mu_B^2}{a_B^3} + \left(b_A - b_{M^\ddagger} + \frac{\bar{\alpha}\epsilon^2}{DrkT} \right) \Gamma + \frac{\Phi_A + \Phi_B - \Phi_{M^\ddagger}}{kT}. \quad (57) \end{aligned}$$

It will be noted that the Debye-Hückel terms have here vanished, so that there should now be a correlation between $\log k_1$ and Γ instead of $\sqrt{\Gamma}$ as in the case of ionic reactions; this has often been observed.⁷¹

Another test of the form of this equation would be to plot the logarithm of the rate constant, extrapolated to zero ionic strength, against the reciprocal of the dielectric constant, selecting reactions in which the dipole moment of the uncharged molecule is zero, and for which the Kirkwood term disappears. According to equation (57) straight lines having slopes equal to

⁷¹ E. g., Brønsted & Wynne-Jones, Trans. Faraday Soc. 25: 59. 1929.

$$\frac{Z_A^2 e^2}{2kT} \left(\frac{1}{r_A} - \frac{1}{r_{M^\ddagger}} \right)$$

should be obtained. Unfortunately, for reactions which have been studied in a range of solvents, data which will allow extrapolation to zero ionic strength are not available. However, the ionic strength effect on reactions of this type is very small, owing to the cancellation of the Hückel and Debye-McAulay terms in (57), and it is therefore

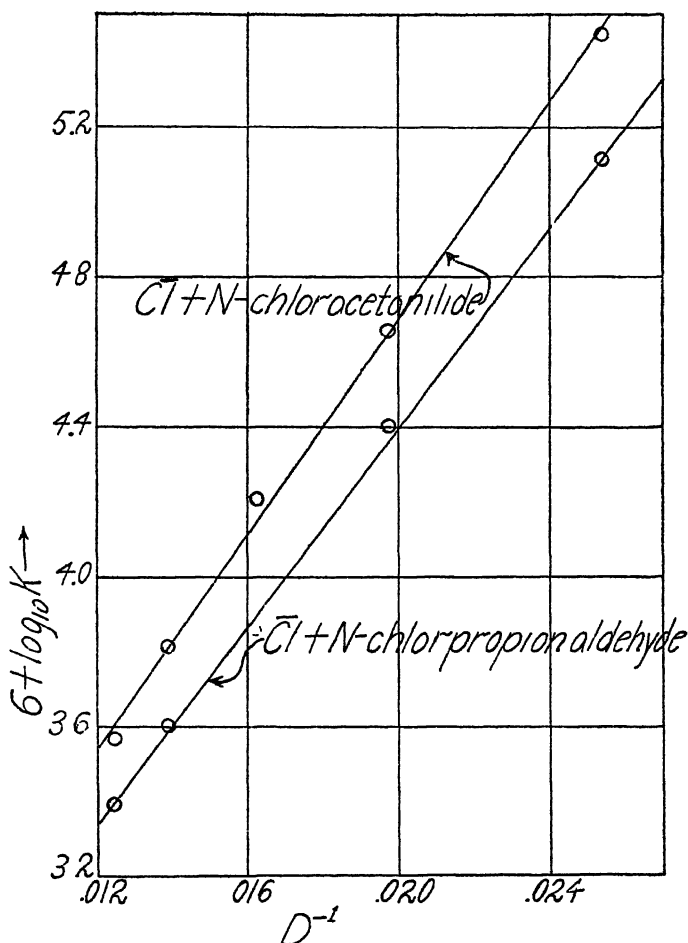


FIGURE 10. The logarithm of the specific reaction rate plotted against the reciprocal of the dielectric constant for the hydrochloric acid-catalysed conversion of N-chloranilides into the p-chloranilides. $T = 25^\circ \text{C}$.

sufficient for the time being to use the ordinary unextrapolated constants, and the dielectric constants of the pure solvents. This has been done in FIGURE 10 for the transformations of N-chloracetanilide and N-chlorpropionanilide into the p-chlor-compounds in the presence of hydrochloric acid,⁷² reactions which may involve an initial attack by the chloride ions. It is seen that good straight lines are obtained.

Summary of Solvent Effects in Reactions Involving Long Range Forces

We may now summarize and elaborate our conclusions of the last three sections with regard to the effect of solvents on reactions in which the electrostatic forces are of primary importance. For purely ionic reactions the slope of the curve obtained by plotting the logarithm of the rate at zero ionic strength against $1/D$ is equal, according to equation (44) to

$$\frac{\varepsilon^2}{2kT} \left[\frac{Z_A^2}{r_A} + \frac{Z_B^2}{r_B} - \frac{(Z_A + Z_B)^2}{r_{M^\ddagger}} \right].$$

the non-electrostatic effects being assumed to be negligible. If the charges on both of the reactants are the same this expression will in all probability be negative, since the radii appear to the first powers, and will therefore have a much smaller effect on its magnitude than the ionic charges which appear to the second powers. We therefore expect the rate of a reaction between ions of the same sign to be increased by an increase in the dielectric constant. It appears that this is always the case; examples are the reactions between thiosulphate and bromoacetate ions, and between bromphenol blue and hydroxyl ions. When however the charges are of opposite sign the slope will be positive, and the rate at zero ionic strength diminished by an increase in the dielectric constant. An example of this is the hydrolysis by hydroxyl ions of the trimethylsulphonium cation. These same qualitative conclusions were pointed out by Scatchard from equation (45).

If the charge on one of the reactants (B) is zero, and this molecule has no dipole moment, the slope is given by equation (57) to be

$$\frac{Z_A^2 \varepsilon^2}{2kT} \left[\frac{1}{r_A} - \frac{1}{r_{M^\ddagger}} \right]$$

This will always be positive, since the radius of the activated complex will be greater than that of the ion A ; moreover the sign is unlikely to

⁷² Fontein, *Rec. Trav. Chim.* **47**: 635. 1928.

be altered even if B is, a dipole since the dipolar effects are small. The rates of reactions of this type will therefore be diminished by increasing the dielectric constant, whatever the charge on A . This is found to be the case for all of the reactions between alkyl halides and hydroxyl, alkoxyl and halide ions. This effect could not be anticipated from equation (45).

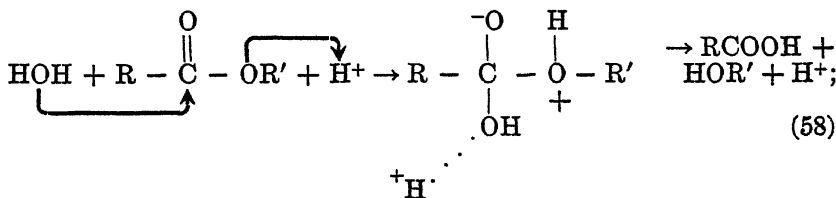
In the case of reactions between dipoles the slope of $\log k_T = 0$ against $(D - 1)/(2D + 1)$ is given by equation (49) as

$$-\frac{1}{kT} \left[\mu_A^2/a_A^3 + \mu_B^2/a_B^3 - \mu_M^2/a_M^{\ddagger 3} \right].$$

This may be positive or negative according as the dipole moment of the activated complex is large or small in comparison with those of the reactants. For reactions in which the final products are ionised it is reasonable to assume that the moment of the complex is large, so that, since $(D - 1)/(2D + 1)$ increases as D increases, reactions of this type should be accelerated by solvents of high dielectric constant. Reactions between amines and alkyl halides, and water hydrolyses of halides, which are reactions in which the products are ionic, are accelerated by such solvents.

The Hydrolysis of Esters

The authors are not aware of any real exceptions to these simple rules, but the acid and alkaline hydrolysis reactions of esters are apparent ones. These are reactions between ions and neutral molecules, and might therefore be expected to be retarded by ionising solvents; in fact they are accelerated,^{73, 74} as is seen from the curves in FIGURE 8. The explanation is clear when the mechanisms of the two reactions are considered; these are, according to the scheme suggested by Lowry⁷⁵ and substantiated by much later work:—
acid hydrolysis:—

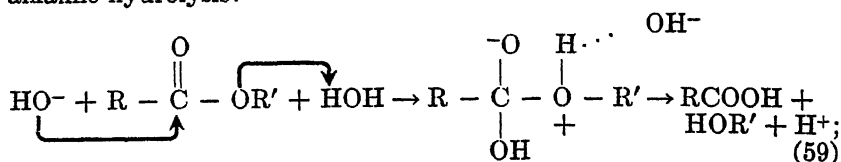


⁷³ Acid hydrolysis, *e. g.* Harned & Samaras, *ref.* (63).

⁷⁴ Alkaline hydrolysis, *e. g.* Fairclough & Hinshelwood, *ref.* (64).

⁷⁵ See Waters, "Physical Aspects of Organic Chemistry", chapter 12. 1935.

alkaline hydrolysis:—



the arrows indicate the movements of the electrons.

The specific rates of the reactions, in terms of the activity coefficients, are therefore:—

acid hydrolysis:—

$$k_1 = \kappa \frac{kT}{h} K_0^\ddagger \frac{\alpha_{\text{H}_2\text{O}} \alpha_{\text{ester}} \alpha_{\text{H}^+}}{\alpha_{\text{complex}}} \quad (60)$$

alkaline hydrolysis:—

$$k_1 = \kappa \frac{kT}{h} K_0^\ddagger \frac{\alpha_{\text{OH}^-} \alpha_{\text{ester}} \alpha_{\text{H}_2\text{O}}}{\alpha_{\text{complex}}} \quad (61)$$

while the actual rates are:—

acid hydrolysis:—

$$v_1 = \kappa \frac{kT}{h} K_0^\ddagger \frac{\alpha_{\text{H}_2\text{O}} \alpha_{\text{ester}} \alpha_{\text{H}^+}}{\alpha_{\text{complex}}} c_{\text{H}_2\text{O}} c_{\text{ester}} c_{\text{H}^+} \quad (62)$$

alkaline hydrolysis:—

$$v_1 = \kappa \frac{kT}{h} K_0^\ddagger \frac{\alpha_{\text{OH}^-} \alpha_{\text{ester}} \alpha_{\text{H}_2\text{O}}}{\alpha_{\text{complex}}} c_{\text{OH}^-} c_{\text{ester}} c_{\text{H}_2\text{O}}. \quad (63)$$

The catalysing effects of the ions are interpreted as usual as being due to the fact that the ions are involved in the reaction; they may also have some effect on the activity coefficients of the activated complexes. The influence of the solvent is interpreted in terms of the hypothesis that the activated complex is even more polar than the reactants, one of which is an ion. Owing to the peculiar structure of the activated complex it is no longer correct to regard it as a spherically symmetrical ion; in particular the hydrogen or hydroxyl ion which is about to be split off when the activated complex decomposes is probably to be regarded as being nearly fully charged in the complex. There is therefore virtually no increase in radius of the (hydrogen or hydroxyl) ion when the complex is formed; in addition an extra separation of charges takes place. The acceleration of the reaction

by ionising solvents is therefore understandable from this point of view.^{75a}

The conclusions of this and the preceding section are collected in the accompanying summary.

Summary of Solvent Effects

Reaction Type	Effect of Increasing Dielectric Constant	Examples
Reaction between two ions:—		
(a) Charges of like sign	Rate increased	$\text{BrCH}_2\text{COO}^- + \text{S}_2\text{O}_3 = \text{OH}^- + (\text{BrPhB})^=$
(b) Charges of opposite sign	Rate diminished	$\text{OH}^- + \text{Me}_3\text{S}^+$
Reaction between an ion and a neutral molecule		
	Rate diminished	$\text{OH}^- + \text{RX}^{76, 77}$ $\text{OR}^- + \text{RX}$ $\text{X}^- + \text{RX}^{78, 79}$ $\text{Me}_2\text{-t-Bu-S}^+ + \text{H}_2\text{O}^{80}$
Reaction between two dipolar molecules with the formation of a polar product		
	Rate increased	$\text{R}_2\text{N} + \text{RX}$ $\text{H}_2\text{O} + \text{RX}$ Acid and alkaline hydrolysis of esters

HEATS AND ENTROPIES OF ACTIVATION

The equilibrium constant K_0^\ddagger in the rate equation (10) may be expressed as a free energy:—

$$K_0^\ddagger = e^{-\Delta F_0^\ddagger/RT} \quad (64)$$

and therefore in terms of a heat and an entropy:—

$$K_0^\ddagger = e^{-\Delta H_0^\ddagger/RT} e^{\Delta S_0^\ddagger/R} \quad (65)$$

ΔF_0^\ddagger , ΔH_0^\ddagger and ΔS_0^\ddagger are the free energy, heat and entropy changes taking place in the process of activation for the reaction proceeding in the infinitely dilute vapor. In order to find the corresponding terms for the reaction in solution the activity coefficients must be expressed as free energies. Thus we define $\Delta \bar{F}^\ddagger_{\alpha_A}$ by

$$\Delta \bar{F}^\ddagger_{\alpha_A} = RT \log \alpha_A \quad (66)$$

^{75a} The authors are indebted to Professor George Scatchard for a discussion of this question.

⁷⁶ De Bruyn & Steger, *Rec trav. chim* 18: 41, 311. 1899.

⁷⁷ Hughes, Ingold & Shapiro, *Jour. Chem. Soc.* 225. 1936.

⁷⁸ Bergmann, Polanyi & Szabo, *Zeit physikal. Chem* B20: 161. 1933.

⁷⁹ Juliusburger, Topley & Weiss, *Jour. Chem. Phys* 3: 437. 1933.

⁸⁰ Hughes & Ingold, *Jour. Chem. Soc.* 1571. 1933.

whence

$$\alpha_A = e^{\Delta \bar{F}^\ddagger \alpha_A / RT} = e^{\Delta \bar{H}^\ddagger \alpha_A / RT} e^{-\Delta \bar{S}^\ddagger \alpha_A / R} \quad (67)$$

Similar expressions are obtained for D_B , D_C , α_M^\ddagger , α_N ,

Introducing these expressions for K_0^\ddagger and the activity coefficients into equation (10), we obtain for the specific rate of the reaction

$$k_1 = \kappa \frac{kT}{h} e^{-\Delta H_0^\ddagger / RT} e^{\Delta S_0^\ddagger / R} \times \frac{e^{\Delta \bar{H}^\ddagger \alpha_A / RT} e^{-\Delta \bar{S}^\ddagger \alpha_A / R} e^{\Delta \bar{H}^\ddagger \alpha_D / RT} e^{-\Delta \bar{S}^\ddagger \alpha_B / R}}{e^{\Delta \bar{H}^\ddagger \alpha_M^\ddagger / RT} e^{-\Delta \bar{S}^\ddagger \alpha_M^\ddagger / R} e^{\Delta \bar{H}^\ddagger \alpha_N / RT} e^{-\Delta \bar{S}^\ddagger \alpha_V / R}} \quad (68)$$

$$= \kappa \frac{kT}{h} e^{-\{\Delta H_0^\ddagger - (\Delta \bar{H}^\ddagger \alpha_A + \Delta \bar{H}^\ddagger \alpha_B + \quad) + (\Delta \bar{H}^\ddagger \alpha_M^\ddagger + \Delta \bar{H}^\ddagger \alpha_N + \quad)\} / RT} \times e^{\{\Delta S_0^\ddagger - (\Delta \bar{S}^\ddagger \alpha_A + \Delta \bar{S}^\ddagger \alpha_B + \quad) + (\Delta \bar{S}^\ddagger \alpha_M^\ddagger + \Delta \bar{S}^\ddagger \alpha_N + \quad)\} / R} \quad (69)$$

so that the actual energy and entropy of activation are given by

$$\Delta H^\ddagger = \Delta H_0^\ddagger - (\Delta \bar{H}^\ddagger \alpha_A + \Delta \bar{H}^\ddagger \alpha_B + \quad) + (\Delta \bar{H}^\ddagger \alpha_M^\ddagger + \Delta \bar{H}^\ddagger \alpha_V + \quad) \quad (70)$$

$$\Delta S^\ddagger = \Delta S_0^\ddagger - (\Delta \bar{S}^\ddagger \alpha_A + \Delta \bar{S}^\ddagger \alpha_B + \quad) + (\Delta \bar{S}^\ddagger \alpha_M^\ddagger + \Delta \bar{S}^\ddagger \alpha_V + \quad). \quad (71)$$

In order therefore to be able to understand the heats and entropies of activation we have to consider the heat and entropy terms associated with the reactants, the activated complex and the intermediates. These terms, according to their definitions by equation (67), are the amounts by which the heats and entropies of the actual solutions under consideration differ from those of ideal solutions (the heat of solution of an ideal solution is of course zero). By making use of the theories of the various types of solutions we may obtain a fair idea of the magnitude of these quantities, and can understand to some extent the effects of solvents on them.

No completely satisfactory treatment of the problem of heats and entropies of solution has however been given, although a fair amount of experimental work has now accumulated. The theories of Rushbrooke and Kirkwood which take into account molecular ordering due to the intermolecular forces do not help much in this respect, since as has already been observed, the contributions to the free energy made by ordering of this type are of a very small order of magnitude. In fact, the deviations of the entropies of solution are

frequently large, and must therefore be attributed to other causes, some of which have recently been discussed by Hildebrand.⁸¹ The most important factors tending to produce order in a solution and in a pure liquid, and therefore to decrease the entropy, are chemical association, dipole orientation, and geometrical shape. The large effects due to the first and second of these in the case of pure liquids are apparent from the large positive deviations from the Trouton and Hildebrand rules found with such liquids as water, ammonia and the alcohols; three different lines of evidence which point to lack of free rotation in such compounds as these have been quantitatively considered.⁸² In addition Hildebrand tabulates a number of entropies of vaporisation of pure liquids clearly show the importance of geometrical shape, in that liquids composed of molecules of unsymmetrical shape have low entropies probably owing to a lack of free rotation. In view of these findings, the results of Scatchard⁸³ that certain liquids mix with an increase of entropy which is greater than that of an ideal solution, are not surprising. Benzene and cyclohexane are an example; benzene molecules are flat and cyclohexane molecules puckered, so that the former can rotate more freely in a mixture of the two than in pure benzene. Such effects as these, however, which are due to abnormalities in the pure liquids, are not our primary concern here, where we have to consider the solutions with reference to the dilute vapors.

An empirical result with regard to heats and entropies of activation has been pointed out by Evans and Polanyi,⁸⁴ and supported by further examples given by Bell,⁸⁵ Butler,⁸⁶ and others. It is that the heats and entropies of solution are often related in a manner expressed by the equation

$$T\Delta S = \alpha\Delta H + \beta \quad (72)$$

where α is always positive and less than unity. A theoretical discussion of the relationship has been given by Bell.⁸⁵ In view of the applicability of equation (72) to the reactants, the intermediates, and the activated complex in a reaction, an analogous linearity between the heats and entropies of activation is to be expected. That this is the case was pointed out by Evans and Polanyi.⁸⁴ Later examples

⁸¹ Hildebrand, *Jour. Chem. Phys.* **7**: 233. 1939.

⁸² Kincaid & Eyring, *Jour. Chem. Phys.* **6**: 620. 1938. Debye, *Physikal. Zeit.* **36**: 193. 1935.

⁸³ Scatchard, Wood & Mochel, *Jour. Phys. Chem.* **43**: 119. 1939.

⁸⁴ Evans & Polanyi, *Trans. Faraday Soc.* **32**: 1333. 1936.

⁸⁵ Bell, *Trans. Faraday Soc.* **33**: 496. 1937.

⁸⁶ E. G. Barclay & Butler, *Trans. Faraday Soc.* **34**: 1445. 1938.

have been given by Fairclough and Hinshelwood,⁶⁴ who, however, interpret the results differently, in that they believe the correlation to be between the entropy (or $\log PZ$) and the reciprocal of the square root of the heat of activation. In FIGURE 11 are plotted their values for the heats and entropies of activation for the alkaline hydrolysis of ethyl benzoate, and it will be seen that the relationship is accurately

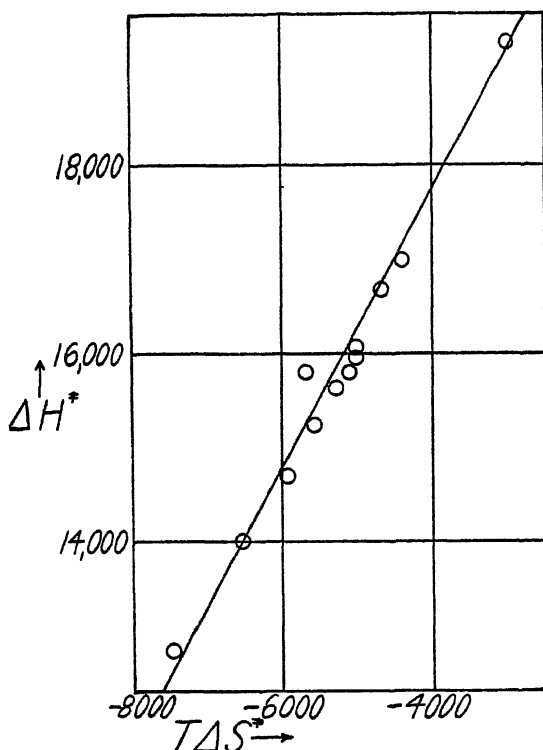


FIGURE 11. Plot of heat of reaction against entropy of reaction for the alkaline hydrolysis of ethyl benzoate in alcohol water mixtures.

held. Deviations from linearity are observed when there is solvation of the reactants or the activated complex, and it would appear that such deviations, and the deviations of the specific rates from their expected relationships with the dielectric constant, run parallel to one another. For example, when the formation of quaternary ammonium salts is studied in solvent mixtures one of the components of which is polar, it is found that the heat of activation is lowered to an abnormal degree as the polar component is added; *i. e.* the lowering

of the heat of activation is greater than corresponds to the change in the entropy of activation. Thus on changing from benzene to nitrobenzene there is always an increase in entropy of activation, and a decrease in the heat of activation.^{87, 88} The extent to which the heat is abnormal must be related to the difference between the heat of solvation of the complex and those of the reactants.

When the solvent is changed changes in entropy appear always to be found. When, however, slight changes in the reactants are made by introducing substituents at some distance from the reacting centers, the position is found to be somewhat different;^{87, 89} in about one half of the accurately studied examples the entropy of activation remains constant in spite of changes in the heat of activation, while in the other half a more or less linear relationship between the two is observed.

⁸⁷ Fairclough & Hinshelwood, *Jour. Chem. Soc.* 1573. 1937.

⁸⁸ Laidler & Hinshelwood, *Jour. Chem. Soc.* 862. 1938.

⁸⁹ Hinshelwood, Laidler & Timm, *Jour. Chem. Soc.* 848. 1938.

DISCUSSION OF PAPER BY LAIDLER AND EYRING (THE EFFECT OF SOLVENTS ON REACTION RATES)*

BY GEORGE SCATCHARD

Since the discussion which follows must be limited to those points upon which I differ from the very interesting paper of Laidler and Eyring, I wish to say at the start that I consider that our differences relate to relatively minor details and that we are in agreement in the important matters, and that many of the apparent differences arise from an inaccurate representation of my theory¹ in their paper.

Although I occasionally used the language of the collision theory, the actual derivations do not depend at all upon that theory. I calculated the concentration of critical complexes directly from the concentration of one reacting species around a molecule of the other. This was the only method available at the time. It has, moreover, the advantage of determining directly the quantity desired instead of using the difference, sometimes small, of two large numbers.

The difference between Laidler and Eyring's treatment of reactions between ions and mine does not lie in my use of collision theory nor in any limitation in my theory that the collision diameter a must be equal to $r = r_A + r_B$. It lies in the difference in models for the critical complex. I assume two spheres with the charge of one of the original ions symmetrically distributed around the center of each; they assume a single sphere with the net charge symmetrically distributed about its center. These are the two extremes, both as to shape and as to charge distribution. Intermediate possibilities are discussed elsewhere.²

It is hard to understand the origin of the misconception that a must equal r in my treatment, for this is definitely discussed as a special case. The fundamental equation is (3) of Ref. 1,

$$C_x = K'' C_A C_B \exp. \left[\frac{-\epsilon^2 Z_A Z_B}{DkT} \cdot \frac{e^{-\kappa r}}{r} \cdot \frac{e^{\kappa a}}{1 + \kappa a} \right]$$

in which C_x is C_M^* of their paper, K'' is K^* and the other symbols have the same significance. This equation is valid only when $r \geq a$. When $r = a$ the equation becomes

* Contribution from the Research Laboratory of Physical Chemistry, Massachusetts Institute of Technology, No. 450

¹ Scatchard, Chem. Rev. 10: 229 1932.

² Scatchard, Jour. Chem. Phys. 7: 657. 1939.

$$C_z = K'' C_A C_B \exp. \left[\frac{-\epsilon^2 Z_A Z_B}{DkT} \left(\frac{1}{r} - \frac{\kappa}{1 + \kappa a} \right) \right].$$

The difference between the two equations is very small unless r is much greater than a .

It should be emphasized in this connection that any use of the parameter a assumes that every ion in the solution has the same radius. If the radii are different there is, strictly speaking, no a for any ion. The equations for two different radii and a discussion of the approximations which can be made for a in the general case, with conclusions but little different from those of Laidler and Eyring, were published some time ago.³ Any errors in these approximations affect the extrapolation to zero ionic strength but not the change with dielectric constant. For solvents of high dielectric constant it is reported⁴ that, "there is no uncertainty in the extrapolation."

In considering the change with changing dielectric constant, it should be kept in mind that the theories of solution give as activity coefficients the ratio of activity to mole fraction and not the ratio of activity to relative volume concentration. The rate constants in terms of concentrations should be corrected to mole fractions by multiplying by $(\Sigma N/V)^{\nu-1}$, in which ΣN is the total number of moles, including solvents, in volume V and ν is the order of the reaction. This correction removes the sudden change of slope for the thiosulfate-bromoacetate reaction in Laidler and Eyring's Figure 3.² The more gradual change at lower dielectric constants can also be removed, or very greatly decreased, by a more exact extrapolation to zero concentration.² However, there are other reactions, which show a similar change of slope which cannot be explained in this way and for which Laidler and Eyring's explanation seems the most probable. The fact that the corrected slope for the thiosulfate-bromoacetate reaction in urea water mixtures, which probably give the most reliable slope, corresponds to $r = 6.4 \text{ \AA}$, which is also the value of a determined for sodium salts, is not a confirmation of my equation. The fact that 6.4 \AA is a reasonable value for r only shows that the experiments do not disprove the theory, for the experiments can also be explained by reasonable values of the three radii in Laidler and Eyring's equation.

An experimental test of the two models is possible when $Z_B = -Z_A$. Laidler and Eyring's equation makes $\log \gamma_A \gamma_B / \gamma_{M^{\pm}}$ equal to $\log \gamma_A \gamma_B$, which can be determined by solubility measurements, while mine makes it equal to

³ Scatchard, *Physikal. Zeit.* **33**: 22. 1932

⁴ LaMer, *Jour. Franklin Inst.* **225**: 709. 1938.

$$[2/(1 + r_A/r_B) (1 + r_B/r_A)] \log \gamma_A \gamma_B.$$

The factor in brackets is $\frac{1}{2}$ if $r_A = r_B$ and smaller if the two radii are different. We may expect the answer to lie between these two extremes.

Laidler and Eyring say of the effect of changing dielectric constant on the reaction of an ion with a neutral molecule, "This effect could not be anticipated from equation (45)." This is quite true, for equation (45) applies only to reactions between ions, but it should be said also that the effect was predicted in my paper.¹ If the reciprocals of the dielectric constant are strictly additive, the logarithm of the rates is a linear function of the reciprocal of the dielectric constant of the solvent, and r is approximately the average of r_A and r_M of Laidler and Eyring, which shows that the two treatments are consistent. However, if it is the polarization which is additive the slope decreases for large values of the dielectric constant, and if the dielectric constant itself is additive the slope actually changes sign.

Professor Eyring has expressed the opinion that the difference between the average dielectric constant and the local dielectric constant around the ions caused by electrical saturation should have a greater effect upon the calculations by my method than upon those by his, and he has asked me to present here my opinion. The qualitative discussion which follows applies equally to the effect of a segregation of one component of a mixed solvent around the reactants or to any other lack of homogeneity.

It is important to recognize that it is not a question of determining whether the difference between my model in an inhomogeneous solvent and my model in a homogeneous solvent of the same macroscopic dielectric constant is greater than the difference between his model in the same two solvents, but is a matter of determining whether the actual reacting system in the inhomogeneous solvent differs more from my model in the homogeneous solvent than it does from his model in the homogeneous solvent. So the effect of the inhomogeneity in either case is that upon the actual system and not that upon the model. If the effect were such that $\log k$ were still a linear function of $1/D$, it would merely change the apparent size determined from either model. Since Eyring's model gives three adjustable parameters and mine only one, his model could absorb a larger effect without yielding an improbable size, but this advantage is trivial. Any change in the slope of $\log k$ vs. $1/D$ or any change of $\log k$ with changing medium but unvariant dielectric constant would be as troublesome for one model as for the other for it would yield a size which varied with the solvent.

My treatment is strictly analogous to the determination of Bjerrum's electrostatic association constant, which Fuoss and Kraus have found to correspond to a constant size over a very large range of dielectric constant.

ACTIVATION ENERGIES IN SOLUTION REACTIONS

By J. C. WARNER

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The purpose of this paper is to discuss recent views on the activation energies of solution reactions. It is now well known that for a specific reaction, the ordinary Arrhenius energy of activation obtained from the variation of the rate constant with temperature (i. e. $2.3 RT^2 (d \log k/dT)$) depends, especially in reactions involving one or more ions, upon the solvent used, upon whether the solvent is one of constant composition or of constant dielectric constant, and upon the ionic strength.

The proper interpretation of these various energies of activation and the relations between them becomes a matter of special importance in any attempt to relate solution kinetics to the kinetics of reactions in gases. In the following, we shall attempt to review the present situation and to indicate the experimental conditions under which one may obtain values of the various quantities of theoretical significance. We also shall apply these views and relationships to experimental data obtained in our laboratory and to the data of other investigators.

The earlier attempts¹ at applying the kinetic equation of the Collision Theory of bimolecular gas reactions to bimolecular reactions in solution were marred by the practice of using an energy of activation obtained under the most diverse experimental conditions and comparing the resulting term representing the collision frequency with a frequency calculated using the ordinary formulas for gases. Considering the diversity of the types of reactions included and the variety of experimental conditions under which activation energies were obtained, it is not surprising that the summary by Moelwyn-Hughes revealed values of $k_{\text{obs}}/k_{\text{calc}}$ which ranged from 10^2 - 10^{-9} .

For further progress, following this method of approach to the problem, it seemed evident that we must: (a) Find, by use of suitable experimental conditions or by suitable methods of calculation from experimental values, an energy of activation which is more nearly comparable to the energy of activation for gaseous reactions; (b) Make a sounder interpretation of the collision frequency term in the kinetic equation for reactions in solution.

¹ Moelwyn-Hughes, *Chem. Rev.* **10**: 241. 1932; "Kinetics of Reactions in Solution". Oxford University Press. 1933.

Our own interest in the subject began just prior to the publication of LaMer's² paper on "The Temperature Dependence of the Energy of Activation.—The Entropy and Free Energy of Activation," in which, using statistical methods, he developed the earlier qualitative idea of Trautz³ that the energy of activation should vary with the temperature if there exists a heat capacity of activation.

$$\frac{\partial \Delta E_a}{\partial T} = \Delta \bar{C}_a = \bar{C}_A - (\bar{C}_A + \bar{C}_B) \quad (1)$$

By analogy to the usual thermodynamic relations, LaMer defined an entropy of activation

$$\Delta S_a = \int \Delta C_p d \ln T = \int \left(\frac{\partial \Delta E_a}{\partial T} \right) d \ln T \quad (2)$$

If then the integrated Arrhenius equation be written

$$\log k = B - \Delta E_a / 2.3RT \quad (3)$$

$$B = \log Z^\circ + \Delta S_a / 2.3R$$

when the activation energy does not vary with temperature, $\Delta S_a = 0$ and $B = \log Z^\circ$. In this paper, LaMer summarized experimental data which indicated that ΔE_a may vary considerably with temperature for reactions in solution. The activation energies used in this summary were obtained in the usual way from the variation of the rate constants with temperature in solvents of fixed composition. To the best of our knowledge, all energies of activation in solution reactions prior to this time had been obtained in solvents of fixed composition. At the time, we were engaged in studies of the influence of the ionic strength and the dielectric constant of the solvent upon the rate of conversion of ammonium cyanate into urea.⁴ The pronounced effect of each of these factors upon the rate caused us to wonder about their influence upon the energy of activation. Any effect of ionic strength may readily be eliminated by using rate constants extrapolated to zero ionic strength (k°) and obtaining an energy of activation from the variation of these constants with temperature. However, when the temperature of a solvent of fixed composition is raised, there are accompanying changes in properties of the solvent which may influence the rate of reactions taking place in the solvent.

² LaMer, *Jour. Chem. Phys.* **1**: 289. 1933.

³ Trautz, *Zeit. anorg. chem.* **106**: 149. 1919. Essex & Gelormini, *Jour. Am. Chem. Soc.* **48**: 882. 1926.

⁴ Warner & Stitt, *Jour. Am. Chem. Soc.* **55**: 4807. 1933. Warner & Warrick, *Jour. Am. Chem. Soc.* **57**: 1491. 1935.

For reactions involving ions or even polar molecules, the dielectric constant is such a property. Therefore when a reaction is studied over a temperature range in a solvent of fixed composition, the rate constants change not only because of the pure temperature effect but also because the dielectric constant of the solvent is decreasing as the temperature is increased.

The above considerations led us to the following inquiries:

- (1) Is the energy of activation obtained in solvents of constant dielectric constant a quantity more nearly comparable to the energy of activation in gaseous reactions than the usual quantity obtained in solvents of fixed composition?
- (2) What relation exists between the energies of activation in solvents of constant composition and solvents of constant dielectric constant?
- (3) Would one find a change in the energy of activation with temperature in solution reactions involving ions if adjustments were made in solvent composition to maintain the same dielectric constant over the entire temperature range?

In gaseous reactions, there is no appreciable change in the environment of the reacting molecules with temperature and the energy of activation is obtained from what might be termed a dependence of the rate constants upon the temperature alone. It therefore seemed logical⁵ that an energy of activation for a solution reaction obtained from

$$(\partial \log k / \partial T)_{xi}, \quad (5)$$

where the xi 's represent all variables other than the temperature which influence the rate, should be the quantity comparable to the energy of activation in the theory of gaseous reactions.

$$\Delta E = 2.3RT^2 (\partial \log k / \partial T)_{xi} \quad (6)$$

It has been demonstrated by our work and by that of many other investigators that the principal variables which influence the rate of ionic reactions are temperature, dielectric constant and ionic strength. As previously mentioned, one may eliminate the influence of ionic strength for the time being by working with rate constants at zero ionic strength (k°).

Assuming that

$$\log k^\circ = f(D, T) \quad (7)$$

we obtain

$$\frac{d \log k^\circ}{dT} = \left(\frac{\partial \log k^\circ}{\partial T} \right)_D + \left(\frac{\partial \log k^\circ}{\partial D} \right)_T \frac{dD}{dT} \quad (8)$$

⁵ Svrbely & Warner, Jour. Am. Chem. Soc. 57: 1883. 1935.

Multiplying both sides by $2.3RT^2$, yields

$$2.3 RT^2 \frac{d \log k^\circ}{dT} = 2.3 RT^2 \left(\frac{\partial \log k^\circ}{\partial T} \right)_D + 2.3 RT^2 \left(\frac{\partial \log k^\circ}{\partial D} \right)_T \frac{dD}{dT} \quad (9)$$

which is a relation between the energy of activation ($\mu = 0$) in a solvent of constant composition and the energy of activation in a solvent of constant dielectric constant.

$$(\Delta E^\circ)_C = (\Delta E^\circ)_D + 2.3 RT^2 \left(\frac{\partial \log k^\circ}{\partial D} \right)_T \frac{dD}{dT} \quad (10)$$

For solvents of fixed composition

$$D = a e^{-b/D} \quad (11)$$

$$\frac{dD}{dT} = -bD \quad (12)$$

The quantity $(\partial \log k^\circ / \partial D)_T$ may always be obtained from experimental studies of the variation of k° with D at constant temperature. For reactions between ions it may be evaluated from the theory of Christiansen and Scatchard⁶ which yields the result

$$\left(\frac{\partial \log k^\circ}{\partial D} \right)_T = \frac{\epsilon^2 Z_A Z_B}{2.3 k D^2 T a_i} \quad (13)$$

Therefore, for reactions between ions

$$(\Delta E^\circ)_C = (\Delta E^\circ)_D - \frac{\tilde{N} b \epsilon^2 Z_A Z_B T}{D a_i} \quad (14)$$

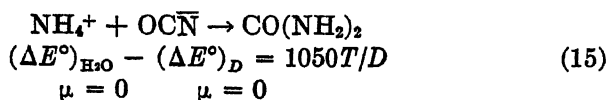
If $Z_A Z_B$ is negative (reaction between ions of unlike sign)

$$(\Delta E^\circ)_C > (\Delta E^\circ)_D$$

but if $Z_A Z_B$ is positive (reaction between ions of like sign)

$$(\Delta E^\circ)_C < (\Delta E^\circ)_D$$

Applied to the conversion of ammonium cyanate into urea



$$(\Delta E^\circ)_{28.3\% \text{ MeOH}} - (\Delta E^\circ)_D = 735 T / D \quad (16)$$

$$\mu = 0 \quad \mu = 0$$

Calculated values of these differences in energies of activation are compared with experimental differences in TABLE 1.

⁶ Scatchard, Chem. Rev. 10: 229. 1932. Christiansen, Zett. physikal. Chem. 113: 35. 1924.

TABLE 1

			Calc.	Expt.
$(\Delta E^\circ)_{\text{H}_2\text{O}}$	—	$(\Delta E^\circ)_D = 63.5$	= 4,860	4,740 cal.
$\mu = 0$		$\mu = 0$		
$(\Delta E^\circ)_{\text{H}_2\text{O}}$	—	$(\Delta E^\circ)_D = 55$	= 4,860	5,080 cal.
$\mu = 0$		$\mu = 0$		
$(\Delta E^\circ)_{23.3\% \text{ MeOH}}$	—	$(\Delta E^\circ)_D = 63.5$	= 4,130	4,210 cal.
$\mu = 0$		$\mu = 0$		

Since the publication of the original paper, Svirbely has continued the study of this reaction in MeOH-H₂O mixtures⁷ down to constant dielectric constants of 35, and in ethylene glycol-H₂O mixtures⁸ to dielectric constants of 40. In all of these studies the above relationships apply and in no case has there been any evidence for a variation in $(\Delta E^\circ)_D$ with temperature.

In a recent investigation which is now ready for publication,⁹ these ideas have been applied to the reaction between ethylene chlorohydrin and hydroxyl ion in water and in constant dielectric constant mixtures of EtOH-H₂O and Dioxane-H₂O. In this example of a reaction between an ion and an uncharged molecule, $(\partial \log k^\circ / \partial D)_T$ might be obtained from the theory of Harned and Samaras.¹⁰ However, due to the approximations in the theory, it is better to take experimental values of $(\partial \log k^\circ / \partial D)_T$ in testing the Svirbely-Warner relation. Here again there is no evidence for any variation of $(\Delta E^\circ)_D$ with temperature, but there is definite evidence of an increase in $(\Delta E^\circ)_{\text{H}_2\text{O}}$ with temperature, and $(\Delta E^\circ)_{\text{H}_2\text{O}}$ may be calculated satisfactorily from $(\Delta E^\circ)_D$ by equation (10). These calculated values are compared to experimental values in TABLE 2.

TABLE 2

Solvent	t°C	Avg. $(\Delta E)_D$ at $D = 74.86$	$(\Delta E)_{\text{H}_2\text{O}} - (\Delta E)_D$ Eq. (10)	$(\Delta E)_{\text{H}_2\text{O}}$ Eq. (10)	$(\Delta E)_{\text{H}_2\text{O}}$ Experimental
EtOH-H ₂ O	0°	20,300	2,480	22,780	$\Delta E_{0-15}^\circ = 22,900$
Dioxane-H ₂ O	0°	20,910	2,000	22,910	
EtOH-H ₂ O	15°	20,300	2,750	23,050	$\Delta E_{15-25}^\circ = 23,210$
Dioxane-H ₂ O	15°	20,910	2,190	23,100	
EtOH-H ₂ O	25°	20,300	2,830	23,130	$\Delta E_{25-35}^\circ = 23,440$
Dioxane-H ₂ O	25°	20,910	2,650	23,560	

⁷ Svirbely & Schramm, Jour. Am. Chem. Soc. **60**: 330 1938.

⁸ Lander & Svirbely, Jour. Am. Chem. Soc. **60**: 1613. 1938.

⁹ Winstrom & Warner, Jour. Am. Chem. Soc. (forthcoming publication).

¹⁰ Harned & Samaras, Jour. Am. Chem. Soc. **54**: 9. 1932.

It is interesting to note that if $(\Delta E)_D$ is independent of the temperature $(\Delta E)_C$ may vary with the temperature. From TABLE 2, it is evident that the experimental variation of $(\Delta E)_{H_2O}$ with temperature is equal within the experimental error, to that predicted by equation (10).

For reactions between ions, one may relate $(\Delta E)_{H_2O}$ to $(\Delta E)_{H_2O}$, and

$$(\Delta E)_D \text{ to } (\Delta E)_D, \\ \mu = 0 \quad \mu = C$$

by differentiating

$$\ln k = \ln k^\circ + \frac{\varepsilon^2 Z_A Z_{BK}}{DkT(1 + A\kappa)} \quad (17)$$

and multiplying by RT^2 . Applied to the ammonium cyanate reaction, one obtains

$$(\Delta E)_{\mu=C} = (\Delta E^\circ)_{\mu=0} + 8.35 \times 10^6 RT^2 \sqrt{\mu} \\ \left\{ \frac{3/2 T^{1/2} D^{3/2} + 100.6 D \sqrt{\mu}}{(T^{3/2} D^{3/2} + 100.6 DT \sqrt{\mu})^2} \right\} \left\{ 1 + \frac{d \ln D}{d \ln T} \right\} \quad (18)$$

For relating $(\Delta E)_D$ to $(\Delta E)_D$ Equation (18) is somewhat simplified

$$\mu = C \quad \mu = 0$$

because $d \ln D / d \ln T = 0$.

Substantial agreement between $(\Delta E)_{\mu=0.44}$ calculated by equation (18) and the experimental values obtained in the conversion of ammonium cyanate into urea is shown by the data in TABLE 3.

TABLE 3

		MeOH-H ₂ O		
		H ₂ O	D = 63.5	D = 55.0
$(\Delta E)_{\mu=0}$		23,580	18,840	18,500
$(\Delta E)_{\mu=0.44}$	Eq. (18)	23,360	19,280	18,950
$(\Delta E)_{\mu=0.44}$	Exptl.	23,240	19,380	18,850

It is interesting to note that, in agreement with the theory for a reaction between ions of opposite sign, an increase in the ionic strength causes a decrease in the activation energy in solvents of constant composition, but causes an increase in the activation energy in solvents of constant dielectric constant.

In the period since the publication of the paper by Svribely and Warner, several interesting and important points of view on solution kinetics have been advanced.¹¹⁻¹⁶ We wish now to examine these views, especially the recent ones advanced by LaMer, from the standpoints of their relation to the ideas of Svribely and Warner and the possibility of experimentally evaluating the various quantities appearing in the theoretical equations.

In recent papers, LaMer has adopted Eyring's kinetic equation

$$k = \frac{\tilde{k}T}{h} K = \frac{RT}{\tilde{N}h} K \quad (19)$$

Where K is the equilibrium constant in the formation of the activated complex from reactants

$$A + B \rightleftharpoons X$$

$$\Delta F_a = \bar{F}_x - (\bar{F}_A + \bar{F}_B) = -RT \ln K \quad (20)$$

hence

$$k = \frac{\tilde{k}T}{h} e^{-\frac{\Delta F_a}{RT}} = \frac{\tilde{k}T}{h} e^{\frac{\Delta S_a}{R}} e^{-\frac{\Delta H_a}{RT}} \quad (21)$$

Neglecting the $P\Delta V$ terms

$$k = \frac{\tilde{k}T}{h} e^{\frac{\Delta S_a}{R}} e^{-\frac{\Delta E_a}{RT}} \quad (22)$$

By comparison with the logarithmic form

$$\log k = B - \frac{\Delta E_a}{2.3RT} = \log \frac{\tilde{k}T}{h} - \frac{\Delta F_a}{2.3RT} \quad (23)$$

$$B = \log \frac{\tilde{k}T}{h} + \frac{\Delta S_a}{2.3R} = \log v + \frac{\Delta S_a}{2.3R} \quad (24)$$

As LaMer properly points out, his earlier definition of the entropy of activation gave

$$B = \log Z^\circ + \frac{\Delta S_a'}{2.3R} \quad (25)$$

At a given temperature, the rate constant of a reaction between ions in a solvent of dielectric constant D and at an ionic strength corresponding to a given value of K (Debye-Hückel Theory) may be re-

¹¹ Eyring, Chem. Rev. 17: 65. 1935; Jour. Chem. Physics, 3: 107. 1935.

¹² LaMer & Kammer, Jour. Am. Chem. Soc. 57: 2662. 1935.

¹³ LaMer, Jour. Franklin Inst. 225: 709. 1938.

¹⁴ Amis & LaMer, Jour. Am. Chem. Soc. 61: 905. 1939.

¹⁵ Moelwyn-Hughes, Proc. Roy. Soc. 155: 308. 1936; A157: 667. 1936.

¹⁶ Bell, Trans. Faraday Soc. 35: 324. 1939.

lated to the rate constant at infinite dielectric constant (k_∞) by the equation

$$\ln k = \ln k_\infty - \frac{Z_A Z_B \epsilon^2 \tilde{N}}{DRT(r_A + r_B)} + \frac{Z_A Z_B \epsilon^2 \tilde{N}}{DRT} \cdot \frac{\kappa}{(1 + \alpha\kappa)} \quad (26)$$

if the dielectric constant is high enough and the ionic strength low enough that the Bronsted-Christiansen-Scatchard equations for the influence of these variables may be applied. LaMer proceeds by multiplying the terms in equation (26) by $-RT$ and by adding $RT \ln kT/h = RT \ln v$ to each side, obtaining

$$\{-RT \ln k + RT \ln v\} = \{-RT \ln k_\infty + RT \ln v\} + \left\{ \frac{Z_A Z_B \epsilon^2 \tilde{N}}{D(r_A + r_B)} \right\} - \left\{ \frac{Z_A Z_B \epsilon^2 \tilde{N} \kappa}{D(1 + \alpha\kappa)} \right\} \quad (27)$$

Each quantity in brackets represents a free energy term and we may write, using LaMer's notation,

$$\Delta F^* = \Delta F_0^* + \Delta F_D^* + \Delta F_{In}^* \quad (28)$$

Each term in ΔF may be divided into the corresponding energy and entropy terms yielding

$$\Delta E^* = \Delta E_0^* + \Delta E_D^* + \Delta E_{In}^* \quad (29)$$

$$\Delta S^* = \Delta S_0^* + \Delta S_D^* + \Delta S_{In}^* \quad (30)$$

ΔE_0^* and ΔS_0^* obviously represent the energy and entropy of activation in the process of forming the complex at $D = \infty$, i. e. in a medium in which all electrostatic effects have vanished. It seems certain that for reactions between ions these terms ΔE_0^* and ΔS_0^* are more nearly comparable to the energy and entropy of activation in gaseous reactions than any similar terms previously defined. It is, therefore, interesting and instructive to compare the kinetic equation for ionic reactions in which these terms are used with the classical collision theory for gaseous reactions. However, before proceeding with this comparison let us consider the other energy and entropy terms.

$$\text{Since} \quad \Delta F_D^* = \frac{Z_A Z_B \epsilon^2 \tilde{N}}{D(r_A + r_B)} \text{ and } \Delta F_{In}^* = - \frac{Z_A Z_B \epsilon^2 \tilde{N} \kappa}{D(1 + \alpha\kappa)} \quad (31)$$

one may find values for ΔE_D^* , ΔS_D^* , ΔE_{In}^* and ΔS_{In}^* by employing the common thermodynamic relations. Both ΔE_{In}^* and ΔS_{In}^* ¹⁷ are functions of the ionic strength and hence vanish in kinetic equations

¹⁷ LaMer, Jour. Franklin Inst. 225: 731. 1938.

involving rate constants (k°) which have been obtained by extrapolating to infinite dilution.

$$\Delta E_D^* = \Delta F_D^* \left\{ 1 + \frac{T}{D} \frac{\partial D}{\partial T} \right\} \quad (32)$$

$$\Delta S_D^* = \frac{\Delta F_D^*}{T} \left\{ \frac{T}{D} \frac{\partial D}{\partial T} \right\} \quad (33)$$

For experiments made in solvents of constant dielectric constant $\Delta S_D^* = 0$ because $\partial D / \partial T = 0$. However, as LaMer has properly said, ΔE_D^* does not vanish but becomes equal to ΔF_D^* . In such measurements

$$\Delta E_D^* = \Delta F_D^* = Z_A Z_B e^2 \tilde{N} / D (r_A + r_B) \quad (34)$$

Thus in our studies of the rate of conversion of ammonia cyanate into urea in solvents of constant dielectric constant

$$(\Delta E)_D = \Delta E_0^* + Z_A Z_B e^2 \tilde{N} / D (r_A + r_B) \quad (35)$$

$\mu = 0$

$$(\Delta S)_D = \Delta S_0^* \quad (36)$$

$\mu = 0$

$(\Delta E^*)_D$ should therefore be a function of the dielectric constant and $\mu = 0$

for the ammonium cyanate reaction, since $Z_A Z_B = -1$, $(\Delta E^*)_D$ is less $\mu = 0$

than ΔE_0^* and should decrease as the dielectric constant of the iso-dielectric solvent mixture is decreased. That the results of Svirbely and Warner, and Svirbely and Schramm in methyl alcohol-water mixtures are in good agreement with equation (35) is evident from a comparison of the experimental and calculated values of $(\Delta E^*)_D, \mu=0$ in TABLE 4 where values of $B = \log v + \Delta S_0^* / 2.3R$ also are given.

TABLE 4

D		∞	63.5	55	50	45	40
$(\Delta E^*)_D, \mu = 0$	Exptl.	(ΔE_0^*)	18,840	18,500	18,110	17,840	17,590
$(\Delta E^*)_D, \mu = 0$	Eq. (35)	21,420	(18,840)	18,440	18,140	17,770	17,320
B		11.80	11.79	11.80	11.71	11.75	11.77

It is noteworthy that B which should depend only on $\tilde{k}T/h$ and ΔS_0^* is essentially constant.

If we return to LaMer's original definition of the entropy of activation, $\Delta S_a'$ should equal zero in these iso-dielectric solvents since there is no evidence for a variation in ΔE_a with temperature and $B = \log Z$ of the classical collision theory or $B = \log PZ$ in the present notation of the English kineticists. If one makes the ordinary collision frequency calculation at 50° C., using $\sigma_{12} = 2.0\text{\AA}$, one obtains $\log Z = 12.29$ which might be increased to 12.9 by making a maximum correction for free space (factor of four). In all the experiments of Svirbely and Warner and of Svirbely and Schramm in methyl alcohol-water mixtures the value of B ranges from 11.71–11.92 with a probable trend toward somewhat higher values as $D \rightarrow \infty$. In a later study of the same reaction by Lander and Svirbely in glycol-water mixtures, B values range from 12.50–13.0. In the latter investigation, the value of B is not entirely independent of the dielectric constant of the iso-dielectric solvent mixture. Nevertheless one may conclude that in view of the approximate character of many of the relationships involved, which in general have neglected specific solvent effects, the rate of conversion of ammonium cyanate into urea is in good agreement with that predicted by the collision theory.

In a similar manner Amis and LaMer have compared their experimental results on the alkaline fading of brom phenol blue with the predictions of the collision theory. Using values of σ_{12} indicated by the kinetic experiments they find their experimental rates are from 2.58 to 6.38 times as fast as predicted by the simple collision theory. Inasmuch as specific solvent and free space corrections have been neglected, the result here must also be considered in good agreement with the collision theory.

Unfortunately, rate measurements in iso-dielectric solvents over a range of temperature have rarely been made and the ideas discussed in this paper cannot be tested on a large number and variety of reactions. However, the success of the method when applied to two reactions, one between ions of like sign and the other between ions of unlike sign, indicates the usefulness of the ideas which have been advanced and suggests that a complete investigation of the kinetics of a reaction involving ions in solution should always include rate measurements over a range of temperature in iso-dielectric media.

PROTOTROPY AND DEUTEROTROPY IN PSEUDO ACIDS

BY SAMUEL H. MARON^{*} AND VICTOR K. LAMER[†]

INTRODUCTION

Among the many reactions which proceed homogeneously in the liquid phase, protolytic reactions occupy a very prominent part. To realize the importance of this type of reaction it is only necessary to recall that in this category are included the various acid-base catalyzed decompositions, isomerizations, and hydrolyses, and that the common processes of ionization and neutralization are the simplest and most elementary types of protolytic reactions. The common denominator in all these reactions is the transfer of a proton, either from an acidic substrate to a basic acceptor, or from an acid donor to a basic substrate.

The interesting aspect of the kinetics of protolytic reactions is the tremendous difference in the rates with which such reactions proceed. These range all the way from the almost instantaneous rates of ionization and neutralization of ordinary acids and bases to the relatively slow and measurable ionizations and neutralizations of the pseudo acids; to the slow decompositions, as that of nitramide in presence of weak bases; to the slow isomerizations and inversions, as that of acetone and sucrose respectively; and to the slow hydrolyses, as that of the various esters. Experiment has shown that there is no one factor to which this wide distribution of rates can be attributed, but that there are many influences of greater or lesser importance, which go to determine the observed rate. Of these possibly the greatest importance is to be ascribed to the structure and type of compounds involved in the reaction, and hence to the basic or acidic character of the reactants. Other factors involved are the medium in which reaction takes place, statistical and steric influences, and, of course, the activation energies and entropies.

To study in a systematic manner the effect of the various variables involved, it is highly desirable to have a reaction which meets the following conditions:

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1. The rate of the reaction should be conveniently measurable.
2. The observed rate should be unquestionably the actual rate of proton transfer.
3. The substrate should be of a type where systematic substitution of various groups is possible.
4. Substrate should be preferably of a type where the acid or basic strength of the substrate itself is determinate.
5. Reaction should proceed in various media by the same mechanism.

The various protolytic reactions to which these criteria may be applied can be classified into two groups: (a) Acid-base catalyses, and (b) ionizations, neutralizations, and their reverse reactions. In the first class, the acid or base involved acts merely as in intermediary in the transformation of a substrate, and is not in any way removed permanently from the sphere of action. The change involved in the reactions of the second class is more "permanent," however. In a neutralization, for instance, both the acid and base are removed simultaneously and at equal rates; there is no regeneration of a reactant during the reaction. Since the primary process involved is the transfer of a proton from an acid to a base, and since such reactions are not subject to the complicating considerations of the function of the acid-base catalysts, kinetic results obtained with reactions of the second type should be simpler to interpret from the standpoint of the proton transfer process.

Nevertheless, despite the greater simplicity, from a theoretical point of view, of reactions of the second class, no measurements are available for such protolytic reactions with ordinary acids and bases; the rates are too rapid to measure. Elucidations of proton transfer are deduced rather from acid-base catalyses of reactions proceeding at rates suitable for convenient study. However, observation of the kinetics of reactions of the second class is highly desirable, and, although impossible at present with ordinary acids and bases, is possible with the substances known as pseudo-acids.

THE NITROPARAFFINES AS PSEUDO ACIDS

A pseudo-acid has been defined by Hantzsch as a substance whose salt formation is a function of time as against the acid whose neutralization is essentially instantaneous. He states, further, that "if the salt formation is a function of time, then it is evidence that the molecule undergoes a change in the salt formation process; it is also evidence for the fact that the undissociated substance and its ions are constitutionally different." Hantzsch's definition can be amplified and

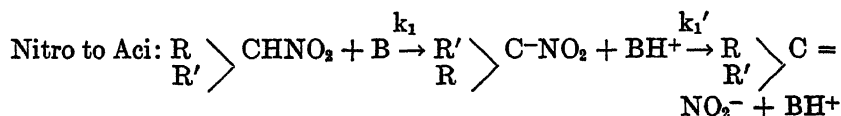
generalized to state that a pseudo acid is an acid whose protolytic reactions are time reactions, as against the very rapid reactions of ordinary acids. Essentially, there is nothing "pseudo" about a pseudo-acid. Hantzsch used the designation because of the relatively high strength of a pseudo acid as against its sluggish action. However, a pseudo-acid must be thought of as being an acid in every sense of the word—with the exception that its protolytic reactions are slower than those of ordinary acids. As Pedersen (1932) stated: "The pseudo acids are not false acids, but acids with false strength. There is no difference in principle between the pseudo- and aci-form. They are both genuine acids, but the pseudo-form is a much weaker acid than the aci-form."

The nitroparaffins, as a class, are pseudo-acids. As is well known, the nitroparaffines exist in two isomeric forms, a nitro- and an aci-form. The two forms differ from each other in several essential respects. The nitro-form dissolves only slightly or not at all in water, reacts slowly with bases and bromine, and shows practically no conductance in water solution. On the other hand, the aci-form dissolves readily in water, reacts extremely rapidly with bases and bromine, and shows appreciable conductance in water solution. The two forms are represented by the structural formulas:



R and R' may be either an alkyl group or a hydrogen atom. The nitro-forms are very weak acids, while the aci-forms are of strengths greater than that of acetic acid.^{1, 2}

The two forms of the nitroparaffines are interconvertible. In presence of bases in general, the nitro-forms are transformed to the aci-forms with rates depending on the strength of the bases employed.^{3, 4} Again in presence of acids in general the aci-forms are transformed into the nitro forms with rates dependent now on the strength of the acids used.⁵ The mechanisms by which these transformations take place can be summarized in the schemes:



¹ Junell, Dissertation, Uppsala. 1935.

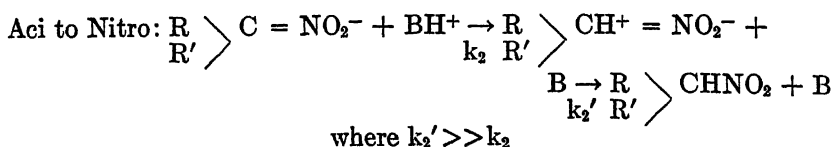
² Maron & Shedlovsky, Jour. Am. Chem. Soc. 61: 753. 1939.

³ Pedersen, Det. Kgl. Videnskab. Selskab. Math.-fys. Medd. 12: 1. 1932.

⁴ Reitz, Zeit. physikal. Chem. A176: 363. 1936.

⁵ Junell, Svensk Kemisk Tidskrift. 46: 125. 1934; cf. ref. 3

where $k_1' \gg k_1$



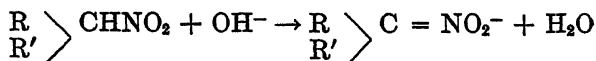
In the first scheme the rate determining step is the proton transfer from the nitroparaffine to the base B , while in the second scheme the rate determining step is the transfer of a proton from the acid BH^+ to the aci-nitroparaffine ion.

The protolytic reactions of the nitroparaffines meet closely the conditions laid down for a desirable reaction to study. It is generally accepted that the measured rates are rates of proton transfer; further, with appropriate methods these rates are more or less conveniently measurable. These reactions offer the possibility of observing proton transfer in ionizations, neutralizations, and acid associations; the possibility of studying effect on the rate of various substituents for R and R' in the molecule; the possibility of observing the effect of medium and statistical factors; and, the possibility of observing and correlating effect of systematic substitution on energies and entropies of activation. Further, it is possible to measure the acid strengths of the various nitroparaffines, and thus gain some ideas of the effect of the acid strength of the substrate on the rate of a protolytic reaction.

Because of the diversity and breadth of the subject, further discussion will be limited primarily to the kinetics of the reaction of the nitroparaffines with hydroxyl and deuteroxyl ions, and to the kinetics of the reverse reaction, i. e., to the reaction of aci-nitroethane ion with the H_3O^+ and D_3O^+ , which have been studied during the past year in the laboratories at Columbia University.

NEUTRALIZATION OF NITROPARAFFINES IN LIGHT WATER

Nitroparaffines in water solution react with hydroxyl ions according to the equation:



The kinetics of this reaction with the substrates nitromethane, nitroethane, nitropropane and nitroisopropane were first studied at 0° by Junell.^{3,5} The extent of reaction at various times was determined by adding to a mixture of nitroparaffine and base after a definite time

interval a definite excess of a mixture of acid and bromine water. The function of the acid was to neutralize the base and thus stop the reaction, while the bromine was to react with the aci ion. From the amount of bromine consumed, the extent of isomerization could be calculated at any elapsed time.

A conductance method for observing the kinetics of these reactions quantitatively was first used in 1934 by Wynne-Jones, who studied the neutralization of nitroethane by $\text{Ba}(\text{OH})_2$ and $\text{Ba}(\text{OD})_2$ at 0° in both light and heavy water. Since the conductance method is more direct, avoids analytical determinations, and is free of the assumptions of the bromination method, it was preferred for the purposes of this research. It depends on the fact that as neutralization proceeds in an equivalent mixture of nitroparaffine and hydroxide, the highly mobile hydroxyl ions are removed to be replaced by the considerably slower anions of the aci-acid formed. Consequently the resistance of the solution increases with time from an initial value, R_0 , to a final limiting value R_∞ , due to the salt of the aci-acid. The concentrations and velocity constants can be calculated in terms of the measured resistances. Details are given by Maron and LaMer.⁶

The procedure employed was to place a definite quantity of the nitroparaffine in water solution in a conductivity cell, and then initiate the reaction by delivering rapidly with a hypodermic syringe an equivalent amount of $\text{Ba}(\text{OH})_2$ solution. The course of the reaction was observed then by taking resistance readings every 15 or 30 seconds, as the occasion demanded.

The second order rate constants of the reaction, which is first order with respect to both nitroparaffine and hydroxyl ion, were evaluated graphically from the resistances in the manner described by Maron and LaMer.⁶ The fidelity with which experimental points followed the expected equation can be judged from FIGURE 1.

The summary of results obtained with nitromethane at 0° , and with nitroethane and nitroisopropane at 0° and 5° is shown in TABLE 1.

These data show that the rate of neutralization falls off rapidly from nitromethane to nitroisopropane. Comparison of the constants with other available data shows that Wynne-Jones' value for nitroethane at 0° agrees well with the value given here, but Junell's results are consistently lower. The explanation for this discrepancy must be sought in Junell's procedure.

The conductance method measures directly the rate of removal of hydroxyl ions. Since the latter are removed in the reaction under

discussion by taking on a proton from the substrate, it can be concluded that the rate of proton transfer to the hydroxyl ion is measured here directly. In Junell's method, however, the rate of proton transfer

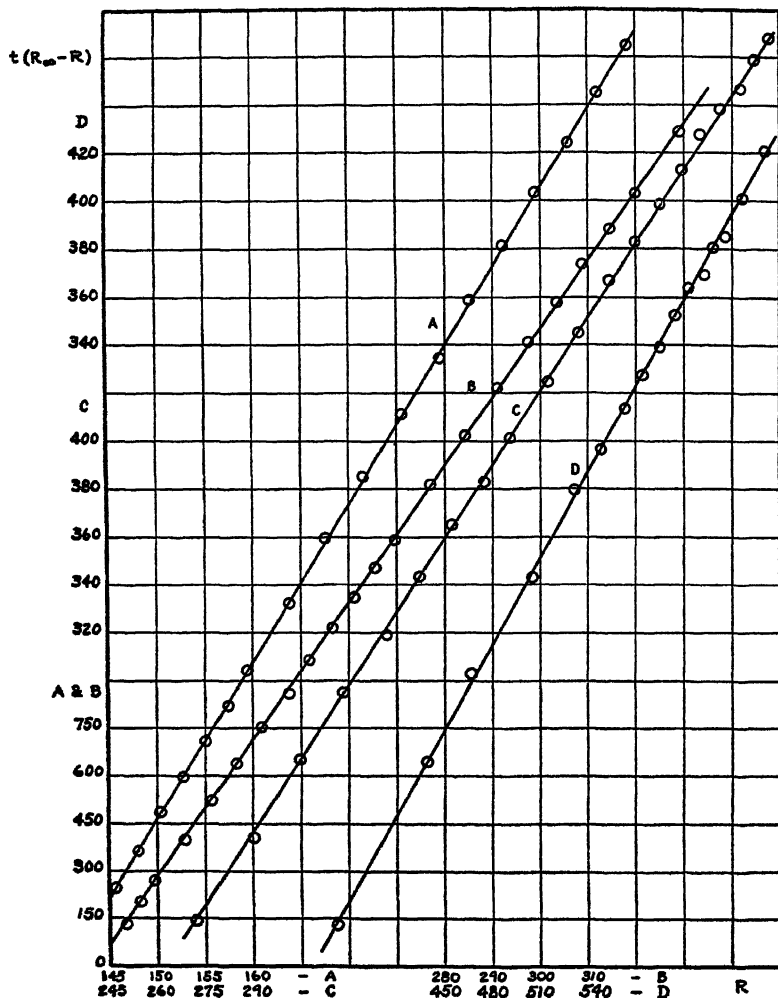


FIGURE 1. Plot of $t(R_{\infty} - R)$ vs. R at 5° . A: 0.0168N nitroisopropane in H_2O ; B: 0.0245N nitroisopropane in D_2O ; C: 0.0273N nitroethane in H_2O ; D: 0.0266N nitroethane in D_2O .

is arrived at indirectly through two fundamental assumptions, (a) that the excess acid added to the reaction mixture would just stop the reaction, and (b) that the bromine added would react very rapidly

with the aci-acid present. There is no question that bromine is absorbed very rapidly by the aci-form of a nitro-paraffine, but just how rapidly is not certain. Two serious objections, however, can be raised to the other assumption. In the first place, in presence of excess mineral acid, the aci nitro-paraffines are unstable.⁷ Secondly, Junell,⁵ and Maron and LaMer⁸ have shown that in presence of H_2O + the anions of the aci-paraffines isomerize rapidly to the nitro forms with rate constants which are 150 for nitroethane and ca. 3000–4500 for nitromethane. It may thus be expected that even with a rapid bromination rate an appreciable back reaction may set in on addition

TABLE 1
SUMMARY AND COMPARISON OF RESULTS ON NITROPARAFFINES IN H_2O

Substrate	Temp.	k_2		
		Maron & LaMer (Cond.)	Junell (Brom.)	Wynne-Jones (Cond.)
Nitromethane	0°	237 6 \pm 1 5	171 \pm 3	—
Nitroethane	0°	39.11 \pm 0 29	35 2 \pm 1 0	39 0 \pm 2 0
	5°	58 37 \pm 0 50	—	—
Nitropropane	0°	—	29.2 \pm 0 2	—
Nitroisopropane	0°	2 08 \pm 0 01	1.94 \pm 0.04	—
	5°	3 36 \pm 0.01	—	—

TABLE 2
COMPARISON OF RESULTS FOR NITROETHANE IN D_2O

	Temp.	k_2	
		Maron & LaMer	Wynne-Jones
First Stage	0°	55 6	60
	5°	81 33 \pm 0 49	—
Second Stage	0°	20 7	36
	5°	29 54 \pm 0 16	—
Third Stage	0°	No result	6–15

of excess mineral acid. The two factors mentioned should operate to give low results for the concentration of aci-form, and hence for k_2 . The error should be especially large for nitromethane, as is actually the case.

The results given in TABLE 1 show that the rate of proton donation by a nitroparaffine to the OH^- ion depends greatly on the nature of

⁷ Nef, *Annalen* 280: 263. 1894.

⁸ Maron & LaMer, *Jour. Am. Chem. Soc.* 61: 692. 1939.

the atoms or groups attached to the alpha carbon. The rate of proton donation is greater the greater the number of hydrogen atoms attached to the alpha carbon, and falls off rapidly as methyl or ethyl groups are substituted for hydrogen. Apparently the length of the substituent alkyl group is of relatively minor importance, since the rates of neutralization of nitroethane and *n*-nitropropane are not very different. On the other hand, the number of alkyl substituents is of importance, as is evidenced by the very large decrease in the rate constants from nitromethane to nitroisopropane.

Again, the decrease in rate from nitromethane to nitroisopropane is not explicable on statistical grounds alone. Instead of a proportionate decrease in rate with decrease in prototropic hydrogens in the substrate, the constants from nitromethane to nitroisopropane are approximately in the ratios 1 : 1/6 : $\frac{1}{120}$.

affected by the difference in the binding forces between the hydrogen and carbon produced by the substitution of alkyl groups.

These results lead to another interesting fact. R. P. Bell⁹ has suggested that Bronsted's relation may be generalized to include the case in which the catalyst remains the same and the substrate varies. He pointed out that for a series of similar substrates the reaction velocity should be related to the acidic or basic strength of the substrate by a relation

$$\begin{array}{ll}
 k_a = G_A K_B^y K_A^x & \text{For acid catalysis} \\
 \begin{array}{ccc}
 & \nearrow & \nwarrow \\
 & \text{substrate} & \text{catalyst}
 \end{array} \\
 k_b = G_B K_A^x K_B^y & \text{For basic catalysis} \\
 \begin{array}{ccc}
 & \nearrow & \nwarrow \\
 & \text{substrate} & \text{catalyst}
 \end{array}
 \end{array}$$

Now, although the isomerism of the nitroparaffines by various bases obeys the Bronsted relation, Bell's more general form of the equation does not hold, for, whereas the velocity constant of nitromethane is greater than that for nitroethane, the ionization constant of the former is smaller than that of the latter, namely, K nitromethane = 2.6×10^{-11} while K nitroethane = 2.7×10^{-9} .

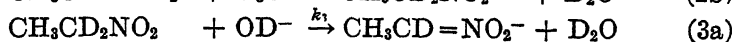
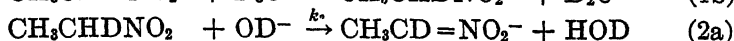
NEUTRALIZATION OF NITROPARAFFINES IN D₂O

Wynne-Jones¹⁰ first called attention to the fact that it should be possible to determine the ratio of proton to deuteron transfer to the

⁹ Bell, Proc. Roy. Soc. London **154A**: 414. 1936; Trans. Faraday Soc. **34**: 229. 1937.

¹⁰ Wynne-Jones, Jour. Chem. Phys. **2**: 381. 1934.

same base and in the same medium by the series of reactions shown below:



The idea of this sequence of reactions is to remove the protons from the α -carbon one at a time with OD^- and reintroduce D atoms with D_3O^+ , until finally only D atoms are present on the α -carbon. Further neutralization should involve then the transfer of a deuteron to the OD^- ion (3a). By this simple artifice it should be possible to measure both the rate of proton transfer (1a) and deuteron transfer (3a) to the same base, OD^- , and in the same solvent D_2O . Reaction (2a) would proceed only as written if the rate of proton removal is considerably greater than that of the deuteron from $\text{CH}_3\text{CHDNO}_2$.

One such sequence of measurements with nitroethane was carried out by Wynne-Jones at 0° . We have repeated the experiment with nitroethane at 0° , and have extended the measurements in D_2O to nitroethane and nitroisopropane at 5° . The results for nitroethane are given in TABLE 2, while the results for nitroisopropane are shown in TABLE 3.

TABLE 3
NEUTRALIZATION OF NITROISOPROPANE IN D_2O AT 5°

99.1% D_2O	
Conc.	k_2
0.0359	4.62
0.0310	4.59
0.0245	4.57
0.0150	4.54
	<hr/>
	4.59 \pm 0.03
	$\pm 0.66\%$

The agreement between Wynne-Jones' value at 0° for the first stage and our result is fair, but there is no agreement at all for the second stage. Wynne-Jones' $k_2 = 36$ is altogether too high in view of the values $k_2 = 20.7$ at 0° and $k_2 = 29.54$ at 5° . For the third stage Wynne-Jones reported a rather unsatisfactory constant varying from 6-15. We could find no satisfactory constants for this stage because

the $\text{CH}_3\text{CD}=\text{NOO}^-$ ion, although stable in the salt, is more sensitive to acid than the corresponding $\text{CH}_3\text{CH}=\text{NOO}^-$ ion, and undergoes some decomposition on addition of D_2SO_4 . On the basis of Nef's work⁷ the decomposition product may be expected to be CH_3CDO . A test with 2, 4-dinitrophenylhydrazine gave a positive reaction. In view of these facts Wynne-Jones' measurements on the third stage are too questionable to have any significance for the calculation of the ratio of proton to deuteron transfer.

A comparison of the rates in H_2O and D_2O shows that the effect of substitution of a CH_3 group for a hydrogen atom is exactly the same in the two media, but that the rate of transfer of a proton from $\text{CH}_3\text{CH}_2\text{NO}_2$ to OD^- in D_2O is 40% greater than the rate of transfer of a proton from the same substrate to OH^- in H_2O . It is doubtful whether the change of solvent from H_2O to D_2O would alone account for a 40% acceleration in rate. It is more probable that the OD^- ion is a stronger base than the OH^- ion, and by virtue of its greater basicity accelerates the rate of proton removal from the substrate.

The activation energies and activation entropies for the various reactions discussed are shown in TABLE 4. In general the activation energies are quite low, and again are lower in D_2O than in H_2O . At the same time, however, the activation entropies are also algebraically lower in D_2O than in H_2O . The importance of the activation entropy

TABLE 4
ENERGIES AND ENTROPIES OF ACTIVATION

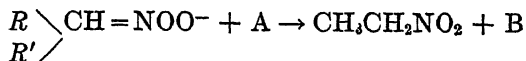
(1) Substrate	(2) Sol- vent	(3) $\log \frac{k_7}{k_0}$	(4) ΔE^* (cal)	(5) B (min. ⁻¹)	(6) $\log k$ (25°)	(7) $-\frac{\Delta E^*}{2.303RT}$	(8) $\frac{\Delta S^*}{2.303R}$	(9) ΔS^*
Nitroethane	H_2O	0 1738	12,100	11 26	1 70	- 9 58	-3 26	-14 99
Nitroethane								
First Stage	D_2O	0 1655	11,500	10 95	1 83	- 9 12	-3 59	-16 41
Second Stage	D_2O	0 1541	10,700	9 89	1 40	- 8 49	-4 65	-21 25
Nitroisopropane	H_2O	0 2082	14,500	11 90	0 42	-11 48	-2 64	-12 06

is clearly brought out from the comparison of the rates and activation energies and entropies for the first and second stages for nitroethane in D_2O . Although the activation energy is lower for the second stage than for the first, still the rate of the former is only about one third of the latter, because the effect of an entropy decrease from the first to the second stages more than outweighs the effect of the decrease in

activation energy. Were it not for the decrease in entropy, the second stage would actually proceed faster than the first.

KINETICS OF REISOMERIZATION OF ACI- TO NITRO-NITROETHANE

Junell¹ showed for nitroethane and nitromethane that in presence of acids in general the aci-nitroparaffine ion reisomerizes to the nitro-nitroparaffine according to the reaction:



The specific rate constants for the various acids used obey the Bronsted relation $\frac{k_a}{p} = G \left(\frac{q}{p} K_A \right)^\alpha$. Since the rate constant for nitroethane in presence of H_3O^+ was not measured by Junell directly, but was inferred from the kinetics of the reaction in buffered solutions in which the hydrogen ion concentration was appreciable, it was decided to measure the rate of the reaction with H_3O^+ in H_2O at 0° and 5° and with D_3O^+ in D_2O at 5° .

The rate of reaction was again followed by conductance. In agreement with Junell we found the rate to be of first order with respect to both the nitroethane and hydrogen ions. The results obtained with nitroethane in light water at 0° and 5° are shown in TABLE 5. The constants obtained from the data are not directly the rate constants of the reaction, but the product of the rate constant k_H^+ and the ionization constant of aci-nitroethane, K_H , where $K_H = \frac{(\text{CH}_3\text{CH}=\text{NOO}^-)(\text{H}_3\text{O}^+)}{(\text{CH}_3\text{CH}=\text{NOOH})}$. This constant was measured electro-

metrically by Maron and Shedlovsky,² and was found to be 4.1×10^{-5} . Hence the rate constants are $k_H^+ = 144$ at 0° and $k_H^+ = 207$ at 5° .

The results of measurements at 5° for the same substrate in D_2O in presence of D_3O^+ are shown in TABLE 6. Now the observed constants are the product of the rate constant k_D^+ and the ionization constant, K_D , of $\text{CH}_3\text{CH}=\text{NOOD}$, or $K_D = \frac{(\text{CH}_3\text{CH}=\text{NOO}^-)(\text{D}_3\text{O}^+)}{(\text{CH}_3\text{CH}=\text{NOOD})}$.

To obtain K_D in D_2O , the graphical relation between the ratio K_H/K_D and $-\log K_H$ given by Rule and LaMer¹¹ was employed. From this plot $K_H/K_D = 3.20$ for an acid of the strength of aci-nitroethane, and hence $K_D = 1.3 \times 10^{-5}$. This value of K_D gives $k_D^+ = 208$ at 5° as against $k_H^+ = 207$ at the same temperature.

¹¹ Rule & LaMer, Jour Am Chem Soc 60:1981 1938.

As far as is known this is the first instance where the rate of transfer of a proton from H_3O^+ to a substrate in H_2O has been found to be the same as the rate of transfer of a deuteron from D_3O^+ to the same substrate in D_2O . In every other case studied¹² with the exception of the mutarotation of glucose, the ratio k_D^+/k_H^+ has been found to be greater than unity. For mutarotation of glucose the ratio is less than unity.¹³

However, the reaction reported here differs in one very important respect from those previously studied. Whereas all the H_3O^+ and D_3O^+ catalyzed reactions studied hitherto involved the transfer of a proton (or deuteron) from H_3O^+ (or D_3O^+) to an uncharged substrate, the reisoimerization of aci-nitroethane involves the transfer of a proton

TABLE 5
KINETICS OF THE REACTION $\text{CH}_3\text{CH}=\text{NOO}^- + \text{H}_3\text{O}^+$ IN H_2O

Exp. No.	α	k_2	K_{HKH^+}
0°			
19-A	0.0383	0.132	0.0054
20-A	.383	.136	.56
26-A	.361	.160	.62
25-A	.361	.163	.63
21-A	.340	.182	.66
27-A	.319	.196	.67
7-A	.289	.205	.64
17-A	.232	.217	.55
22-A	.218	.230	.55
23-A	.218	.240	.57
24-A	.217	.236	.56
8-B	.203	.252	.56
24-B	.180	.290	.59
			0.0059 ± 0.0004
5°			
74-A	0.0328	0.250	0.0088
53-A	.279	.294	.88
72-A	.257	.282	.78
51-A	.249	.325	.88
75-A	.232	.363	.91
73-A	.191	.396	.83
76-A	.138	.510	.78
			0.0085 ± 0.0004

¹² Reitz, Zeit. Electrochem. 44: 72. 1938.

¹³ Hamill & LaMer, Jour. Chem. Phys. 4: 395. 1936

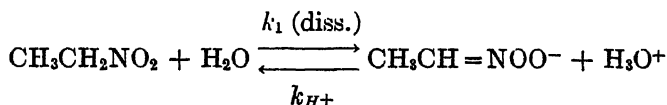
TABLE 6
KINETICS OF THE REACTION $\text{CH}_3\text{CH}=\text{NOO}^- + \text{D}_3\text{O}^+$ IN D_2O

Exp. No.	a	k_2	$K_D k_D^+$
5°			
55-A	0.0259	0.119	0.0032
46-A	233	.127	31
59-A	189	.122	24
56-A	189	.123	24
48-A	186	.156	31
57-A	166	.152	27
58-A	148	.149	23
			0.0027 ± 0.0003

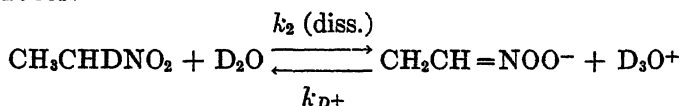
(or deuteron) to the anion of aci-nitroethane at the double bond. Once the proton (or deuteron) is transferred, the attendant electron shift stabilizes the final product, $\text{CH}_3\text{CH}_2\text{NO}_2$ (or $\text{CH}_3\text{CHDNO}_2$).

Since the electron shifts in the nitroparaffines are considered to be very rapid compared to the prototropic changes (Maron and LaMer)⁶ the measured rates of proton and deuteron transfer to the aci-nitroethane ion are the rates of association of the ions of the two acids, $\text{CH}_3\text{CH}_2\text{NO}_2$ and $\text{CH}_3\text{CHDNO}_2$ respectively. The equality of association rates for the proto and deuterio acid observed raises the interesting and important question whether the association rates for all proto and their corresponding deuterio acids are the same, or whether the instance of nitroethane represents an isolated case. The importance of this question is apparent from the following considerations.

If the dissociation constant for the reaction:



be defined as K_1 , then $K_1 = \frac{k_1 (\text{diss.})}{k_{H+}}$. Similarly, if the dissociation constant for:



be defined as K_2 , then $K_2 = \frac{k_2 (\text{diss.})}{k_{D+}}$. For the ratio of dissociation

where K_N and K_A are the ionization constants of the nitro form of the nitroparaffine and the acid A respectively. Knowing K_N , K_A , and k_A , both the rate constants k_B and the equilibrium constants K can be calculated.

The available data for this purpose for nitromethane and the results of calculations are shown in TABLE 7. For nitromethane Junell found that $K_N = 2.6 \times 10^{-11}$. In column (1), (2), and (3) are given

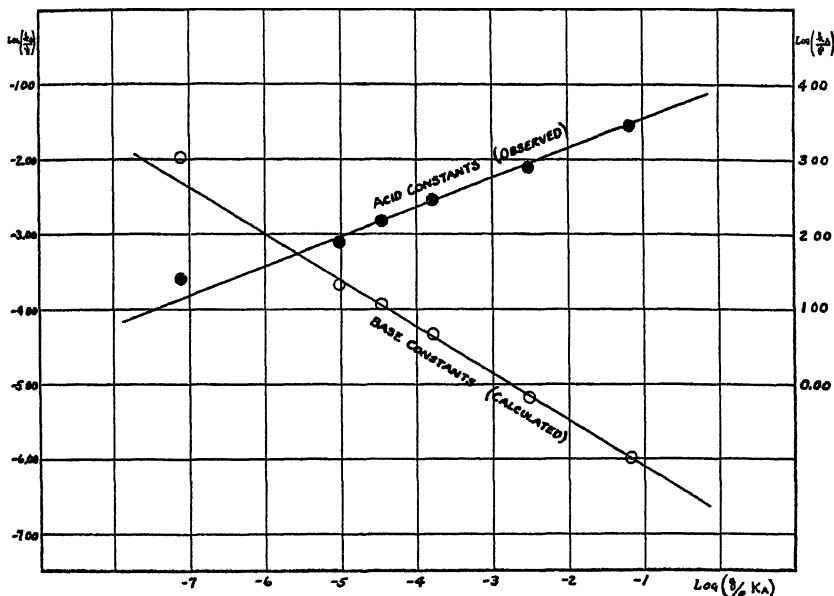


FIGURE 2. Plot of $\log(k_A/p)$ and $\log(k_B/q)$ vs. $\log(q/p K_A)$ for nitromethane at 0°C

Junell's results for the acid reaction. In column (4) is given the equilibrium constant K calculated from K_N/K_A , while in column (5) are given the k_B values calculated from $Kk_A = \frac{K_N k_A}{K_A}$. Column (6)

shows k_B values extrapolated from available data to 0° . The agreement is as good as can be expected. Columns (7) and (8) show values of K and K_A calculated from observed data. These are to be compared with column (4) and (2) respectively. Again the agreement is good.

Since the acid constants obey the Brönsted relation, it is to be expected that the basic constants would do likewise. That this is so is shown in FIGURE 2. From this plot $G_A = 8,200$, $G_B = 1.98 \times 10^{-7}$,

TABLE 7
CALCULATIONS ON NITROMETHANE

1	2	Temp.: 0.0° C.		4	5	6	7	8	9	$K_N = 2.6 \times 10^{-11}$					13
		3								10	11	12			
Acid	K_A obs.	k_A obs.	$K_{calc.} = \frac{K_N}{K_A}$	k_B calc.	k_B obs.	$K_{obs.} = \frac{k_B \text{ obs.}}{k_A \text{ obs.}}$	$K_A \text{ calc.} = \frac{K_N k_A}{k_B}$	Base		p	$q \log \left(\frac{k_B}{q} \right)$	$\log \left(\frac{q K_A}{p} \right)$			
H_2O^+	55.5	4500(?)	4.96×10^{-13}	2.24×10^{-9}	1.4×10^{-9} (Extr.)	3.11×10^{-18}	83.6	H_2O		1	1	-8.65	1	74	
CH_3Cl-CH_2COOH	3.32×10^{-3}	2800	7.83×10^{-6}	2.12×10^{-4}	—	—	—	CH_3COO^-		1	2	-5.98	1	18	
CH_2Cl-CH_2COOH	1.53×10^{-3}	800	1.70×10^{-8}	1.36×10^{-6}	1.1×10^{-9} (Extr.)	1.38×10^{-4}	1.88×10^{-2}	$CH_2Cl-COO^-$		1	2	-5.17	1	51	
CH_3Cl-CH_2COOH	8.0×10^{-4}	290	3.25×10^{-7}	9.43×10^{-6}	—	—	—	$CH_3Cl-CH_2COO^-$		1	2	-4.33	1	80	
CH_3COOH	1.70×10^{-4}	150	1.53×10^{-4}	2.30×10^{-4}	2.1×10^{-4} (Extr.)	1.40×10^{-4}	1.86×10^{-5}	CH_3COO^-		1	2	-3.94	1	47	
$COO^-(CH_2)_2COOH$	2.4×10^{-4}	80	1.07×10^{-5}	8.56×10^{-4}	—	—	—	$COO^-(CH_2)_2-COO^-$		1	4	-3.67	1	02	
$(CH_3)_2AsOOH$	5.7×10^{-7}	210	—	—	—	—	—	$(CH_3)_2AsOO^-$		1	2	—	1	94	
$H_2PO_4^-$	4.1×10^{-4}	50	6.34×10^{-4}	3.17×10^{-2}	—	—	—	HPO_4^{2-}		2	3	-1.98	2	11	
H_2O	2.1×10^{-17}	1.91×10^{-4} (Calc.)	1.24×10^6	—	237.6	—	—	OH^-		1	1	+2.38	1	16.7	

TABLE 8
CALCULATIONS ON NITROETHANE

Temp.: 0.0° C.										
1	2	3	4	5	6	7	8	9	10	11
Acid	K_A obs.	k_A obs.	K calc.	k_B calc.	Base	p	q	$\text{Log} \left(\frac{q}{p} K_A \right)$	$\text{Log} \left(\frac{k_B}{q} \right)$	$\text{Log} \left(\frac{k_A}{p} \right)$
H_3O^+	55.5	150	4.86×10^{-11}	7.3×10^{-9}	H_2O	1	1	1.74	-8.14	2.18
$\text{CH}_3\text{Cl}-\text{COOH}$	1.53×10^{-3}	3.8	1.77×10^{-6}	6.7×10^{-6}	$\text{CH}_2\text{Cl}-\text{COO}^-$	1	2	-2.51	-5.47	0.58
$\text{CH}_2\text{Cl}-\text{CH}_2-\text{COOH}$	8.0×10^{-5}	1.62	3.38×10^{-3}	5.46×10^{-5}	$\text{CH}_2\text{Cl}-\text{CH}_2-\text{COO}^-$	1	2	-3.80	-4.56	0.21
CH_3COOH	1.7×10^{-6}	0.67	1.59×10^{-4}	1.06×10^{-4}	CH_3COO^-	1	2	-4.47	-4.28	-0.174
$\text{COO}^--(\text{CH}_2)_2-\text{COOH}$	2.4×10^{-6}	0.42	1.12×10^{-3}	4.71×10^{-4}	$(\text{CH}_2)_2(\text{COO}^-)_2$	1	4	-5.02	-3.55	-0.377
$(\text{CH}_3)_2\text{As OOH}$	5.7×10^{-7}	0.99	4.74×10^{-3}	4.69×10^{-3}	$(\text{CH}_3)_2\text{As OO}^-$	1	2	-5.94	-2.63	-0.004
H_2PO_4^-	4.1×10^{-3}	0.18	6.59×10^{-2}	1.18×10^{-2}	H PO_4^{1-}	2	3	-7.11	-2.41	-1.046
H_2O	2.1×10^{-17}	3.1×10^{-7}	1.28×10^8	39.1 (obs.)	OH^-	1	1	-16.7	1.59	
										(calc.)

(calc.)

while $\alpha = 0.39$ and $\beta = 0.62$. According to theory, if the calculations are correct $\alpha + \beta$ should equal unity. Actually $\alpha + \beta = 0.39 + 0.62 = 1.01$.

Similar calculations for nitroethane are shown in TABLE 8. Although no experimental data are available for comparing calculated and observed values of k_B , still a test can be made graphically through the

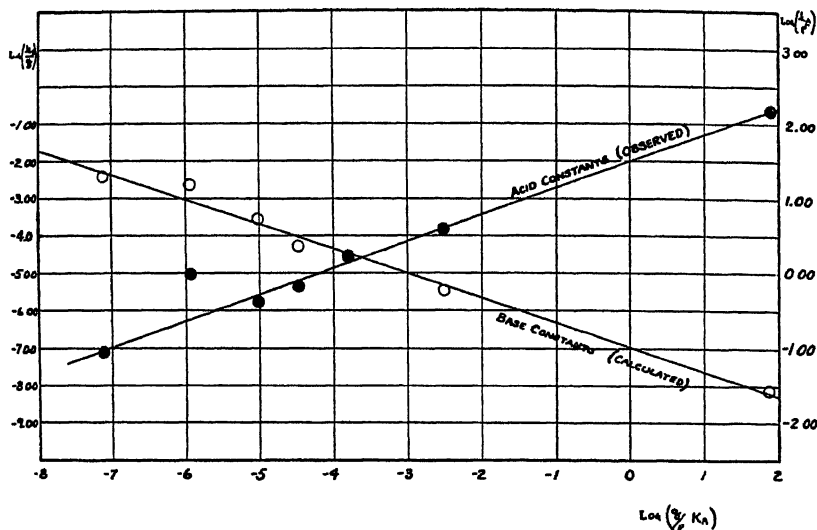


FIGURE 3. Plot of $\log (k_A/p)$ and $\log (k_B/q)$ vs. $\log (q/p K_A)$ for nitroethane at 0°C .

Brønsted relation. The plot is shown in FIGURE 3. Again the expected plot is obtained. For nitroethane $G_A = 32$, $G_B = 1.05 \times 10^{-7}$, $\alpha = 0.36$, $\beta = 0.65$, and $\alpha + \beta = 0.36 + 0.65 = 1.01$.

In closing, attention should be directed to one other relation which stems from the above considerations. Since $k_A/p = G_A \cdot \left(\frac{q}{p} K_A\right)^\alpha$ and $\frac{k_B}{q} = G_B \left(\frac{p}{q} K\beta\right)^\beta = G_B \left(\frac{q}{p} K_A\right)^{-\beta}$, a ratio of one to the other yields:

$$\log \frac{k_A}{k_B} = \log K = \log G_B/G_A - \log K_A = \log K_N - \log K_A$$

$$\therefore K_N = G_B/G_A$$

Applying this relation to nitromethane and nitroethane we get for G_B/G_A :

$$G_B/G_A = \frac{1.98 \times 10^{-7}}{0.82 \times 10^4} = 2.4 \times 10^{-11} \text{ for nitromethane, as against the observed } K_N = 2.6 \times 10^{-11}$$

$$\text{and } G_B/G_A = \frac{1.05 \times 10^{-7}}{32} = 3.3 \times 10^{-9} \text{ for nitroethane as against the observed value of } K_N = 2.7 \times 10^{-9}$$

Hence the ratio G_B/G_A is the ionization constant of the substrate involved in the basic reaction.

KINETICS OF THE EXCHANGE OF OXYGEN BETWEEN ORGANIC COMPOUNDS AND WATER

BY IRVING ROBERTS

From Columbia University, New York

INTRODUCTION

In 1936, there was made available by fractional distillation¹ comparatively large quantities of water ranging in heavy oxygen content from 0.080 to 0.850 mol per cent of H_2O^{18} . It thus became possible to investigate in detail the exchange of oxygen between water and a number of organic compounds. It was soon found that in contrast to deuterium exchange which in many cases takes place at rates which are almost instantaneous, almost all oxygen exchange reactions proceeded at measurable rates. In addition, these exchange reactions seemed to exhibit catalysis by acids and bases.

Kinetics of oxygen exchange have been investigated in only two cases, namely, the acetone-water exchange done by Cohn and Urey,² and the benzoic acid-water exchange studied by Roberts and Urey.³ However, a large number of qualitative exchange experiments have been performed, and for a summary of such data, the reader is referred to the review paper by Reitz.⁴ The results of such work may be summed up with the statement that the only organic compounds which have been found to exchange their oxygen with that of water are aldehydes, ketones, carboxylic acids and a few very reactive alcohols.

METHODS OF ANALYSIS

Before taking up a discussion of the kinetic data, it would be well to present the methods for determining the O^{18} content of a substance. Of the two available methods, the first consists of converting the compound to water and determining the density of the water by a sensitive apparatus such as that of Gilfillan.⁵ The objections to this are two-fold. In the first place, it requires extreme purification of the water without introduction of extraneous oxygen, which may not always be successful when organic impurities are present, and which would be

¹ Huffman & Urey, *Ind. Eng. Chem.* **29**: 531. 1937.

² Cohn & Urey, *Jour. Am. Chem. Soc.* **60**: 679. 1938.

³ Roberts & Urey, *Jour. Am. Chem. Soc.* **61**: 2580. 1939.

⁴ Reitz, *Zeit. Electrochem.* **45**: 100. 1939.

⁵ Gilfillan, *Jour. Am. Chem. Soc.* **56**: 406. 1934.

time consuming in any case. Secondly, the range of such a density apparatus may not be great enough to allow convenient measurement without loss of precision. For example, the maximum density which could be measured on the float apparatus at Columbia University was about 20 parts per million heavier than normal water, with a precision of 1 part per million. In order to measure the water used in much of this work (approximately 370 parts per million heavier than ordinary water), it was necessary to dilute about 20 times, thus introducing an error of 20 parts per million.

The second method available, the use of the mass spectrometer, also presented difficulties. It is not convenient to measure the isotopic ratio of oxygen in the form of water because it would necessitate baking out the instrument after each analysis. Nor is it advisable to place oxygen gas in the instrument, since it reacts with the filament. However, carbon dioxide gas does not offer such difficulties, and it was found suitable to measure the isotopic ratio of oxygen in this form.

Cohn and Urey² devised an equilibration method, whereby the O^{18} content of water could be determined in carbon dioxide. This was accomplished by distilling a known amount of the recovered water under vacuum into a sample tube with a break seal on it, and adding 16 c. c. of carbon dioxide at atmospheric pressure. The mixture was frozen with liquid air and sealed off in vacuum. The sample tube was then shaken vigorously at room temperature until equilibrium was reached. The time necessary to reach the complete equilibrium, $CO^{16}O^{18} + H_2O^{18} \rightleftharpoons CO^{18}O^{18} + H_2O^{16}$, under these conditions, was determined experimentally. TABLE 1 shows that there was no change after three hours, but all samples were actually shaken for five hours to ensure equilibrium.

TABLE 1

Time of Shaking, Hrs.	$CO_2^{18}/CO^{16}O^{18}$
1	359
2	505
3	548
4	550
Ordinary CO_2	237

The $\%O^{18}$ in the water may be calculated from the equilibrium equation, since the measured value in carbon dioxide, the number of moles of water and of carbon dioxide equilibrated are known. The value of the equilibrium constant of this reaction has been found by Weber³ to be 1.047 at 0° . The tank carbon dioxide, always used as

³ Weber, Urey & Wahl, Jour. Chem. Phys. 3: 129. 1934

standard in the analyses, was found experimentally to have the same isotopic concentration before and after equilibration with water, which indicated that the tank carbon dioxide previously had been brought to equilibrium with water. Since all measurements of the isotopic composition of the carbon dioxide were made relative to this standard, the equilibrium constant was implicitly taken into account by using the ratio 250 for $\text{CO}_2^{16}/\text{CO}^{16} \text{O}^{18}$ in the standard carbon dioxide, corresponding to the value 500 reported for the relative abundance of $\text{O}^{16} : \text{O}^{18}$ by the Committee on Atoms of the International Union of Chemistry.⁷ There is very little loss of precision due to dilution of the concentration of O^{18} in this method, since the number of moles of carbon dioxide used is very small compared to the number of moles of water and there is only a small difference between the calculated isotopic composition of the water before equilibration with carbon dioxide and the isotopic composition of the carbon dioxide actually measured. The precision of such mass spectrometer determinations is about 1%.

KINETICS OF THE ACETONE-WATER EXCHANGE

The exchange of oxygen between acetone and water was studied by Cohn and Urey in a medium consisting of 90% acetone and 10% water, the heavy oxygen content of the water ranging from 0.08% to 0.75% O^{18} . The method of recovering the water for analysis was as follows: a 14 c. c. sample was chilled in a bath at dry ice temperature where the water crystallized out from the mixture. The acetone was then drawn off through a fritted glass filter and the water remaining was washed twice with 10 c. c. portions of benzene to remove any remaining acetone. In this way, from 0.5 to 0.7 c. c. of water was recovered for analysis from each sample. The water was then shaken with carbon dioxide and the carbon dioxide analyzed as described above.

In 0.0001 N HCl at 25° the reaction was found to be too fast for measurement, and the same was true for 0.001 N NaOH. Therefore, all the experiments were done with salicylate buffers. The values of the hydrogen-ion concentration were determined from a series of pH measurements of these solutions.

The rate expression for the reaction $\text{H}_2\text{O}^{16} + \text{R}_2\text{CO}^{18} \rightleftharpoons \text{H}_2\text{O}^{18} + \text{R}_2\text{CO}^{16}$ was derived by setting up the usual bimolecular equation

$$dx/dt = k(A - x)(B - x) - (C + x)(D + x)$$

⁷ Aston, Bohr, Hahn, Harkins & Urbain, *Rev. Sci. Instruments* 7: 334. 1936.

where $A = (\text{H}_2\text{O}^{16})$ $B = (\text{R}_2\text{CO}^{18})$ $C = (\text{H}_2\text{O}^{18})$ $D = (\text{R}_2\text{CO}^{16})$

initially, and $x =$ concentration of H_2O^{18} formed after time t . The values of k for the forward and reverse reactions are assumed to be identical, since from the experimental data, the value of the equilibrium constant cannot be distinguished from 1.

Since the total amount of O^{18} in the reaction mixture does not change,

$$\frac{C + x_{\infty}}{C + x_{\infty} + A - x_{\infty}} = \frac{B - x_{\infty}}{B - x_{\infty} + D + x_{\infty}}$$

or
$$AB - CD = (A + B + C + D) x_{\infty}$$

Combining these equations and integrating,

$$kt = \frac{1}{A + B + C + D} \ln \frac{x_{\infty}}{x_{\infty} - x}$$

It can be seen that the reaction should be unimolecular in character, and that a plot of $\log (X_{\infty} - X)$ against time should give a straight line. This was found to be the case for all experiments and the slope of the least squares line was used for calculating velocity constants. The concentrations of O^{18} in acetone were assumed to be normal, i. e., 0.200 atom per cent O^{18} .

The results of all the experiments performed given in TABLE 2 and the following discussion of the results are largely taken from the paper of Cohn and Urey.

TABLE 2

Expt.	Initial (H_2O^{18}) m/l.	$C_{\text{Acid}} \times 10^3$	$C_{\text{Salt}} \times 10^3$	$\text{CH}_3\text{X} \times 10^3$	$k/c \times 10^3$	$k \times 10^4$
1	0 005259	1 25	0 125	6 00	1 16	1 49
2	005259	1 25	125	6 00	1 17	1 50
3	005388	9 93	1 04	7 26	6 13	7 87
4*	005233	1 25	0 125*	7 60	1 40	1 80
5	02486	10 38	1 01	7 81	6 31	8 08
6	004855	5 03	1 00	3 82	3 06	3 92
7	004855	10 08	5 15	1 74	5 31	6 80
8	004855	1 97	0 975	1 54	1 17	1 50
9	04059	1 90	9 92	0 21	1 11	1 43

* Concentration of KCl, 0.873×10^{-2}

Experiments 1 and 2 were identical in all conditions except that the zero points were taken at slightly different times. The values of the constants are 1.49×10^{-4} and 1.50×10^{-4} , respectively. When the

concentrations of both the acid and the salt were increased (experiment 3) approximately by a factor of 8, the constant increased to 7.87×10^{-4} . In order to determine how much of this increase was due to an increase in ionic strength, experiment 4 was run with the same concentrations of salicylic acid and sodium salicylate as were used in experiments 1 and 2, but enough potassium chloride was added to make the total salt concentration identical with that of experiment 3. The rate constant in experiment 4 was 1.80×10^{-4} , as compared with 1.50×10^{-4} for experiments 1 and 2 and 7.87×10^{-4} for experiment 3; this comparison indicates that the primary salt effect is relatively small, if not zero. Experiment 5 was a check of experiment 3; the initial (H_2O^{18}) concentrations were 0.02486 and 0.005233 m./l. respectively. Since the percentage of O^{18} in the water in experiment 5 was greater than in the acetone, the net reaction proceeded in the opposite direction from the previous experiments.

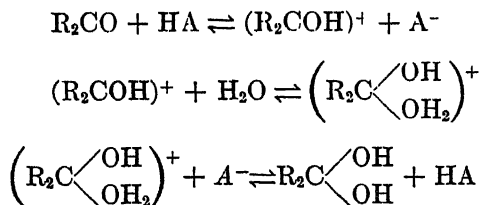
In experiments 3, 6 and 8, the salt concentration was held constant, and the acid concentration was varied approximately in the ratio 10 : 5 : 2. In experiments 3 and 7, the acid concentration was held constant, and the salt concentration was varied in the ratio of 1 : 5. The effect of varying the salt concentration is even more obvious in experiments 8 and 9. In these experiments the contribution of the hydrogen ion catalysis is small, the acid concentration is held constant, and the salt concentration is varied from 1 to 10 and the rate changes from 1.50×10^{-4} to 1.43×10^{-4} .

If all these results are combined and the values of the catalytic coefficients, k_a , k_b , and k_{H^+} , for the acid, base and hydrogen ion, respectively, are determined by the method of least squares from the nine equations of the form $k = c_a k_a + C_{H^+} k_{H^+} + c_b k_b$ where k is the observed over-all reaction rate constant, each result being weighted to give a minimum percentage error, the following values are obtained: $k_a = 0.0682$, $k_b = 0.000150$, $k_{H^+} = 116.8$. It is obvious that the catalytic constant for the salicylate ion is negligible, and on the assumption that k_b is zero, the values become: $k_a = 0.0683$, $k_{H^+} = 116.5$.

It was thus established that the exchange reaction between acetone and the oxygen of water is catalyzed by hydrogen ion, hydroxyl ion, and the salicylic acid molecule, but not by salicylate ion. The fact that salicylate ion does not catalyze the reaction does not, of course, exclude the possibility of observing basic catalysis if stronger bases than salicylate ion were used. However, a preliminary investigation showed that in the presence of secondary phthalate ion, a much stronger base, the catalysis is very small, if not zero.

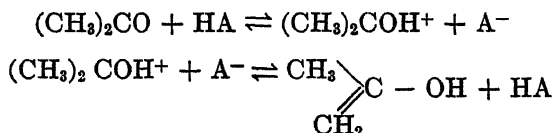
MECHANISM OF THE ACETONE-WATER EXCHANGE

The mechanism considered by Cohn and Urey to be the most probable for the acid-catalyzed exchange is the following:



Following these three steps, all the reactions must be reversed in order to return to acetone with an exchanged oxygen. Only the first step or the third step could be rate controlling, since only these will give the observed general acid catalysis.

If this mechanism is correct, the rate of this reaction is significant in interpreting the mechanism of the enolization of acetone. Pederson⁸ has shown the following to be by far the most probable mechanism for the acid-catalyzed enolization:



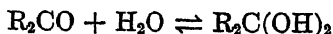
Either of these two steps could be rate controlling for both would give general acid catalysis.

The present exchange reaction affords a method of distinguishing which of these steps must be the slower. It can be seen that the first reactions of both mechanisms are identical. Now, as nearly as could be estimated by Cohn and Urey, the hydrogen ion catalyzed enolization reaction is slower than the oxygen exchange reaction by a factor of 500. This implies that the rate of the first step in the exchange (and hence in the enolization) is at least 500 times as great as the rate of enolization itself. Of necessity, then, the second step of the enolization mechanism (proton removal) is rate controlling.

It should be mentioned, however, that several alternative mechanisms for acetone exchange are possible; this will be seen from the discussion of the benzoic acid exchange mechanism given below.

A further conclusion to be drawn from this work is that the rapid and reversible formation of an acetone hydrate in one step

⁸ Pederson, *Jour. Phys. Chem.* **38**: 581. 1934.



is not possible. If such were the case, the oxygen exchange of acetone would not only be rapid, but also uncatalyzed by acids or bases.

KINETICS OF THE BENZOIC ACID-WATER EXCHANGE

Benzoic acid exchanges both its oxygen atoms with that of water, the reaction being catalyzed by acids, as is the case for the exchange of carboxylic acids in general^{9, 10, 11}. The usual method for following exchange, that of mixing comparable amounts of the reacting substances, cannot be used at ordinary temperatures in the case of benzoic acid since the compound is not sufficiently soluble in water to cause an appreciable change in the heavy oxygen content of the water. Rather than introduce the complication of a mutual solvent, a method of determining the heavy oxygen content of benzoic acid was devised and the kinetic experiments were carried out in dilute aqueous solution.

EXPERIMENTAL

Decarboxylation Apparatus

The determination of the O¹⁸ content of benzoic acid depends upon the observation of Sabatier and Mailhe¹² that benzoic acid will be converted almost quantitatively into benzene and carbon dioxide when passed over reduced copper at 500°. For this work, it was necessary to construct an apparatus which would perform the decarboxylation of a few milligrams of benzoic acid in a stream of nitrogen gas, and which would be free of traces of oxygen, carbon dioxide, water, or copper oxide. In addition, after each conversion, the apparatus had to be easily made ready for the succeeding one, since many samples were to be converted for each kinetic experiment.

The apparatus finally used is shown in FIGURE 1. Tank nitrogen was admitted at the left through a flowmeter (A) and successively through a tube of copper at 350° (B), a bubbler containing sulfuric acid (C), and tubes D and E, filled with potassium hydroxide and anhydrous calcium sulfate, respectively. At this point the nitrogen can be made to take one of two paths: if stopcock K is closed, it can

⁹ Roberts, *Jour. Chem. Phys.* **6**: 294. 1938.

¹⁰ Senkus & Brown, *Jour. Org. Chem.* **2**: 569. 1938.

¹¹ The present work shows that the statement of Koizumi & Titani (*Bull. Chem. Soc. Japan* **13**: 607. 1938) that only one oxygen atom of benzoic acid is readily exchangeable cannot be correct. Such a statement is quite unreasonable in the light of our present knowledge of the rapid dissociation of the hydrogen ion from a carboxylic acid and of the symmetrical structure of a carboxylate ion.

¹² Sabatier & Mailhe, *Compt. Rendu* **159**: 219. 1914.

pass through *J* into the decarboxylation tube (*I*) containing copper at 500° and into the air through stopcock *F*. On the other hand, with stopcock *J* closed, the nitrogen could enter tube *I* through stopcock *F* and pass successively through *K*, a trap (*L*), and into the air through stopcock *O*. Tube *I* was made of temperature-resistant glass (Corning 172) and sealed directly to the pyrex tubing at either side. Tube *II* was 1.5 cm. in diameter and contained a ring seal with an opening of about 2 mm. diameter. The apparatus also contained a safety (*G*), a manometer (*M*), a sample tube fitted with a break seal (*N*), and a trap (*P*) surrounded with solid CO₂ to protect a Hyvac pump connected at the right.

After sweeping out with hydrogen gas to reduce the oxides of copper,

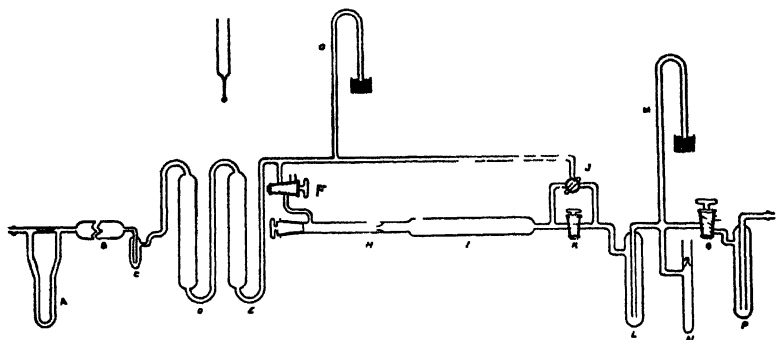


FIGURE 1

the entire apparatus was evacuated and filled with nitrogen. Trap *L* was surrounded with liquid N₂. The nitrogen stream was then adjusted to 30 c. c./min. and directed through *J*, through tubes *I* and *II* and into the air through *F* (stopcocks *K* and *O* being closed). The plug in tube *H* was removed, the tube containing the benzoic acid inserted around the ring seal and the plug replaced. The benzoic acid sample tubes which were 10 mm. in diameter and of the shape shown in the upper left of FIGURE 1, were first wiped with chamois to remove any organic material which would produce carbon dioxide on being heated. The purpose of the inner seal of tube *H* was to direct the stream of nitrogen into the benzoic acid sample tube when it was put into place.

After sweeping out the air introduced with the tube for four minutes, the nitrogen stream was reversed by turning stopcock *F*, closing stopcock *J*, opening stopcock *K*, and opening stopcock *O* to

the air. The benzoic acid was then sublimed into *I* with an ordinary Bunsen flame, both the benzene and the carbon dioxide produced being caught in trap *L*. After allowing the nitrogen stream to continue for fifteen minutes, stopcocks *O* and *K* were closed, and the gas was again directed through *J*, *I*, *H*, and *F*, into the air. The nitrogen gas remaining in the portion of the apparatus to the right of stopcocks *J* and *K* was then pumped out through *O* and *P*. Stopcock *O* was turned off and the liquid nitrogen around trap *L* was removed and replaced with solid carbon dioxide-acetone mixture, thus keeping the benzene frozen while allowing the carbon dioxide to vaporize. After observing the pressure on the manometer (*M*), the carbon dioxide was distilled into the sample tube (*N*) by cooling it to liquid nitrogen temperature, the sample tube being then sealed off at the side-arm and set aside for mass spectrometer analysis.

To prepare the apparatus for the next conversion, the next benzoic acid sample was inserted, stopcock *O* was opened to the air and a new carbon dioxide sample tube sealed on. The air was then pumped out, liquid nitrogen placed around trap *L*, and the evacuated space filled with nitrogen through stopcock *J* (stopcock *F* having been closed). In this way no air was admitted to tube *I* at any time. The nitrogen stream was again directed from left to right through tube *I* and the process carried out as before. The time elapsed between the beginning of one conversion to the beginning of the next was about 35 minutes.

Heavy oxygen benzoic acid was prepared as follows: a solution of 1.7 grams of benzoic acid in 65 c. c. of heavy oxygen water which was also 0.1 N in hydrochloric acid was allowed to stand in the thermostat at 80° for 48 hours. The heavy oxygen water and the hydrochloric acid were completely distilled away in vacuo, and the remaining solid was powdered and dried over phosphorous pentoxide. Conversion of a sample to carbon dioxide by the above procedure and analysis in the mass spectrometer showed that this benzoic acid contained 0.465% O^{18} . Ordinary benzoic acid was found to contain 0.204% O^{18} .

Kinetic measurements were made in an oil thermostat set at 80.00 \pm 0.03° with a Bureau of Standards platinum resistance thermometer. In general, 10 c. c. of hydrochloric acid solution (ordinary H_2O or H_2O^{18}) were pipetted into a 20 c. c. long-necked flask fitted with a ground glass joint and stopper. After allowing the flask to stand in the thermostat for at least thirty minutes, a glass bucket containing about 120 mg. of benzoic acid (ordinary or heavy) was lowered into it by means of a platinum wire. The flask was shaken vigorously and

the time recorded: the benzoic acid was completely dissolved within one minute.

At known intervals, approximately 1 c. c. portions were withdrawn by means of a long medicine dropper and delivered into the benzoic acid sample tubes of the type mentioned above and shown in the upper left of FIGURE 1. The tubes were immediately chilled to room temperature, causing most of the benzoic acid to crystallize and effectively stopping the reaction. Most of the solution was then drawn off by means of a fine capillary pipette, the benzoic acid crystals being large enough to remain behind. Before decarboxylation, the last traces of water were pumped away from each sample by inserting the tube into a larger tube fitted with a ground cap and connected to a Hyvac pump.

The efficiency of this drying process was tested by crystallizing ordinary benzoic acid from heavy oxygen water in the absence of hydrochloric acid catalyst, drawing off the water, and pumping for various lengths of time. The benzoic acid was then decarboxylated and the carbon dioxide analyzed in the mass spectrometer. It was found that four hours of pumping were necessary to remove the water completely within the precision of analysis. About one-fourth of the benzoic acid was also lost during the pumping process.

The carbon dioxide samples from the benzoic acid decarboxylations were analyzed for the ratio of mass 44 to mass 46, corresponding to the species CO_2^{16} and $\text{CO}^{16}\text{O}^{18}$, respectively. The relation of the heavy oxygen content of the carbon dioxide to this ratio can be seen from the following: if α is the atom fraction of O^{18} in the carbon dioxide, the amounts of the three species CO_2^{18} , $\text{CO}^{16}\text{O}^{18}$ and CO_2^{16} will be in the ratio $\alpha^2 : 2\alpha(1 - \alpha) : (1 - \alpha)^2$, assuming the gas to be in statistical equilibrium. The ratio of mass 44 to mass 46 will then be

$$R = \frac{(1 - \alpha)^2}{2\alpha(1 - \alpha)}$$

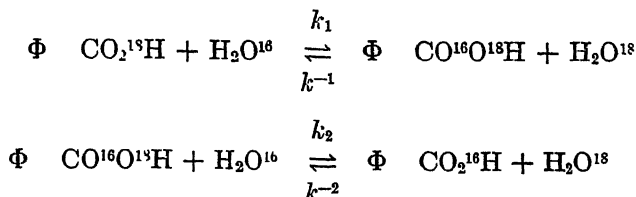
Solving for α we have

$$\alpha = \frac{1}{2R + 1}$$

as the formula for calculating the mol fraction of O^{18} from the experimentally determined ratio.

KINETICS OF THE REACTION

In the exchange of O^{18} between benzoic acid and water, the three species of benzoic acid and two species of water react as follows:



Assuming that there are no specific differences in reactivity between O^{16} and O^{18} species of the same compound, and that both oxygens of benzoic acid exchange with equal ease, the relations of the various rate constants to each other may be determined by inspection. For example, at equal concentrations, reaction 1 will be twice as successful as reaction 2, since only half the reactions of $\Phi \text{ CO}^{16}\text{O}^{18}\text{H}$ with H_2O^{16} will result in exchange; that is

$$k_2 = k_1/2$$

In like manner, it will be seen that $k - 1 = k_1/2$ and that $k_{-2} = k_1$.

If the three forms of benzoic acid are statistically distributed, their concentrations may be represented:

$$[\Phi \text{ CO}_2^{13}\text{H}] = \alpha^2 [\Phi \text{ CO}_2\text{H}]$$

$$[\Phi \text{ CO}^{16}\text{O}^{18}\text{H}] = 2\alpha (1 - \alpha) [\Phi \text{ CO}_2\text{H}]$$

$$[\Phi \text{ CO}_2^{16}\text{H}] = (1 - \alpha)^2 [\Phi \text{ CO}_2\text{H}]$$

where α is the atom fraction of O^{18} in the benzoic acid and $[\Phi \text{ CO}_2\text{H}]$ is the (constant) total concentration of the benzoic acid in mole/liter. Similarly, we may write

$$[\text{H}_2\text{O}^{18}] = \beta [\text{H}_2\text{O}]$$

$$[\text{H}_2\text{O}^{16}] = (1 - \beta) [\text{H}_2\text{O}]$$

where $[\text{H}_2\text{O}]$ is the total concentration of water and β is the atom fraction of O^{18} or the mol fraction of H_2O^{18} in the water.

Considering the initial condition in which the benzoic acid contains the excess of O^{18} , the rate of the reaction will be

$$\begin{aligned} \frac{-d}{dt} \left\{ [\text{O}^{18}] \text{ in benzoic acid} \right\} &= \frac{-d}{dt} \left\{ [\Phi \text{ CO}^{16}\text{O}^{18}\text{H}] + 2 [\Phi \text{ CO}_2^{18}\text{H}] \right\} \\ &= k_1 [\Phi \text{ CO}_2^{18}\text{H}] [\text{H}_2\text{O}^{16}] + k_2 [\Phi \text{ CO}^{16}\text{O}^{18}\text{H}] [\text{H}_2\text{O}^{16}] \\ &\quad - k_{-1} [\Phi \text{ CO}^{16}\text{O}^{18}\text{H}] [\text{H}_2\text{O}^{18}] - k_{-2} [\Phi \text{ CO}_2^{16}\text{H}] [\text{H}_2\text{O}^{18}] \end{aligned}$$

Substituting for the various rate constants their equivalents in terms of k_1 and for the concentrations of reactants the values given above, we obtain, after cancelling terms

$$-2[\Phi \text{ CO}_2\text{H}] \frac{d\alpha}{dt} = k_1 [\Phi \text{ CO}_2\text{H}] [\text{H}_2\text{O}] (\alpha - \beta)$$

$$\frac{d\alpha}{dt} = k (\beta - \alpha), \text{ where } k = k_1 [\text{H}_2\text{O}]/2.$$

Therefore the change of the O^{18} content of the benzoic acid with time should be independent of the concentration of benzoic acid and first order in the difference of O^{18} content of the reactants. Integrating, holding β constant¹³

$$k = \frac{2.303}{t} \log \frac{\alpha - \beta}{\alpha_0 - \beta}$$

By an entirely similar derivation, it can be shown that when the water contains the excess of O^{18} initially

$$k = \frac{2.303}{t} \log \frac{\beta - \alpha_0}{\beta - \alpha}$$

Velocity constants were obtained from the slope of the least squares line obtained by plotting $\log (\alpha - \beta)$ against time. The results of the five experiments performed are shown in TABLE 3. A comparison of Experiments 1 and 2 will show that the rate of the reaction is independent of which reactant contains the excess of O^{18} initially. In Experiment 3, the hydrochloric acid concentration was increased fivefold, causing a fivefold increase in the rate. Decreasing the concentration of benzoic acid by a factor of two in Experiment 4, had no effect on the reaction velocity, as predicted by our derivation above. Finally, addition of potassium chloride in Experiment 5 had no effect on the rate, demonstrating the absence of any salt effect. The variation of k_H is within the precision of about 4% for the determination of a rate constant.

The fact that the exchange reaction is first order in hydrogen ion serves to demonstrate the similarity of the nature of this reaction to the acid catalyzed esterification and ester hydrolysis reactions.

¹³ Under the conditions of these experiments, the water is in large excess over the benzoic acid so that the heavy oxygen content of the water does not change measurably. If the water were not in large excess, one would proceed, as in the case of the acetone exchange, by noting that the total O^{18} concentration is a constant, i. e.

$$\text{O}^{18} = 2\alpha [\Phi \text{ CO}_2\text{H}] + \beta [\text{H}_2\text{O}] = \text{constant}.$$

Substituting for β in the above rate expression,

$\frac{d\alpha}{dt} = \frac{k_1}{2} \left\{ [\text{O}^{18}] - \alpha ([\text{H}_2\text{O}] + 2 [\Phi \text{ CO}_2\text{H}]) \right\}$, an equation which is symmetrical in the water and the benzoic acid and which is easily integrated.

TABLE 3
 RATES OF EXCHANGE AT 80° C.

Expt.	α_0	β	[HCl]	[Φ CO ₂ H]	k	k_H^{**}
1	0.00204	0.00515	0.0932M	0.1088M	0.00129	0.0138
2	0.00465	0.00200	0.0936	0.1058	0.00129	0.0138
3	0.00465	0.00200	0.478	0.1082	0.00693	0.0145
4	0.00465	0.00200	0.478	0.0564	0.00711	0.0149
5*	0.00465	0.00200	0.0936	0.1009	0.00131	0.0141

* This reaction mixture was also 0.0937N in potassium chloride.

** k_H is the rate constant for 1N hydrochloric acid.

$$\text{Mean } k_H = 0.0142 \pm 0.0004$$

The agreement of the rates of these three reactions with each other offers further evidence towards this conclusion. It has already been demonstrated that in methyl alcohol as a medium, the rates of esterification and exchange of benzoic acid are comparable^{14, 15}. In addition, the rate of hydrolysis of methyl benzoate at 80° is of the same order of magnitude as the rate of exchange determined in the present work,¹⁶ the exchange being about ten times faster.¹⁷

The mechanism of benzoic acid exchange is intimately bound up with the mechanisms of the similar ester hydrolysis and esterification reactions. Therefore, the mechanisms of all three reactions will be considered in the following section.

THE MECHANISMS OF ACID CATALYZED ESTER HYDROLYSIS, ESTERIFICATION AND OXYGEN EXCHANGE OF CARBOXYLIC ACIDS

In addition to the known kinetic data on the acid hydrolysis of esters and on the esterification of carboxylic acids, we now have available the results of heavy oxygen studies of both the esterification¹⁸ and the exchange of carboxylic acids. Roberts and Urey¹⁹ were able to show that by combining these data, it was possible to eliminate all but a few of the large number of mechanisms which may be proposed for all three reactions.

¹⁴ Roberts & Urey, Jour. Am. Chem. Soc. 60: 2301. 1938.

¹⁵ Herbert & Lauder (Trans. Faraday Soc. 34: 1219. 1938; Nature 142: 954. 1938) have predicted this result on the basis of previous experiments (ref. 9).

¹⁶ Bolin, Zeit. Anorg. Chem. 177: 227. 1938.

¹⁷ The observed k_H for the exchange was multiplied by 2 to obtain $k_1 \text{H}_2\text{O} = 0.0284$ as compared to $k \text{H}_2\text{O} = 0.0028$ calculated from the data of Bolin for the hydrolysis. k_1 as defined in the derivation above is the constant which should be used for this comparison since it represents the rate constant for the exchange which is effective at every reaction.

¹⁸ Roberts & Urey, Jour. Am. Chem. Soc. 60: 2391. 1938.

¹⁹ Roberts & Urey, Jour. Am. Chem. Soc. 61: 2584. 1939.

The three reactions show a number of striking similarities. Besides the structural similarity of reactants and products, the reactions are all catalyzed by acids. In addition, there seems to be little doubt that both the hydrolysis and esterification are general acid catalyzed. Dawson²⁰ has shown that the hydrolysis of ethyl acetate is catalyzed by acetic acid molecules, chloroacetic acid molecules and bisulfate ions. Hinshelwood²¹ has demonstrated catalysis of the esterification by a number of fatty acid molecules and Goldschmidt²² has done the same for such acids as trichlorobutyric acid. On the basis of this general acid catalysis the retardation of esterification in alcoholic media by the addition of water discovered by Goldschmidt²³ is explained by the fact that ROH_2^+ is a stronger acid than H_3O^+ in the Bronsted sense. The exchange of oxygen between benzoic acid and water is first order in hydrogen ion; as yet there is no data to determine whether the reaction is general acid catalyzed.

A further correlation lies in the fact that the rates of the three reactions are of the same order of magnitude. The nearness of the rates of hydrolysis and esterification is well known; one of the problems in studying the kinetics of either reaction has been the elimination of the reverse one. Furthermore, in a methyl alcoholic medium containing about five per cent water, the rate of esterification is about twice the rate of exchange of benzoic acid;¹⁸ when these rates are corrected to the same concentrations of methanol and water, the latter is about four times as fast as the former. Finally, in water as a medium, the rate of exchange of benzoic acid is about ten times the rate of hydrolysis of methyl benzoate at the same temperature, as shown previously.

A similarity in the linkages broken on esterification and exchange is also known. In the esterification of benzoic acid a carbon-oxygen bond of the benzoic acid is broken rather than that of the methyl alcohol.¹⁸ The same must of course be true for the exchange of oxygen between benzoic acid and water. A recent heavy oxygen study of acid catalyzed ester hydrolysis has shown that the same linkage is broken in this reaction.²⁴

Because of the above evidence, it can be assumed that the mecha-

²⁰ Dawson & Lowson, *Jour. Chem. Soc.* 2444 1927; 393. 1929 Dawson, Pycock & Spivey, *Jour. Chem. Soc.* 291. 1933.

²¹ Rolfe & Hinshelwood, *Trans. Faraday Soc.* 30: 935 1934. Newling & Hinshelwood, *Jour. Chem. Soc.* 1357. 1936. Fairclough & Hinshelwood, *Jour. Chem. Soc.* 593 1939.

²² Goldschmidt, *Trans. Faraday Soc.* 24: 662. 1928.

²³ Goldschmidt, *Ber.* 28: 3224. 1895. Goldschmidt & Udby, *Zeit. physikal. Chem.* 60: 728 1907.

²⁴ Datta, Day & Ingold, *Jour. Chem. Soc.*, 838 1938.

nisms of all three reactions are entirely similar. This would presuppose a symmetrical mechanism for esterification and hydrolysis, since it is reasonable to assume that the reverse of one would be the mechanism of the other. The term "symmetrical mechanism" is used here in the sense that the reverse of such a mechanism for esterification will be a mechanism for hydrolysis which is structurally similar to it. A symmetrical mechanism must obviously be the case for the oxygen exchange reaction, where for example the mechanism of the reaction of $\text{RCO}^{16}\text{O}^{18}\text{H}$ with H_2O^{16} , would be similar to its reverse, the reaction of $\text{RCO}^{16}_2\text{H}$ with H_2O^{18} . Finally, as a further simplification, the usual assumption is made that each mechanism will contain only one rate controlling step, *i. e.* that there is only one activated complex whose disappearance controls the rate of the reaction.

An important consideration which does not seem to have been previously emphasized is that the esterification and hydrolysis reactions are very probably first order in alcohol and water, respectively. The existence of the relationship

$$\frac{\text{mole ester} \times \text{mols water}}{\text{mols alcohol} \times \text{mols acid}} = K,$$

which is so universal as to be largely independent of medium as well as temperature²⁵ would argue at least at equilibrium for a first order dependence of esterification rate on the alcohol concentration and of hydrolysis rate on the water concentration. While some reactions are known to change in mechanism while approaching equilibrium, this would not be expected to take place for these comparatively simple reactions. This is especially true for the exchange reaction, where the equilibrium constant is undoubtedly very close to unity. It is therefore difficult to see how the first order dependence on the alcohol and water concentration would apply to the condition of equilibrium and not to other conditions.

In addition, a first order dependence of the rate of esterification on the alcohol concentration is known in formic and acetic acid as media from the cryoscopic measurements of Kailan and co-workers²⁶ who have also demonstrated different esterification rates for different alcohols.

THE REACTIVE COMPLEX

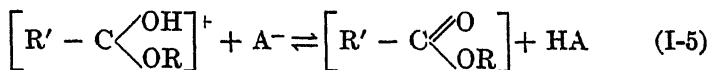
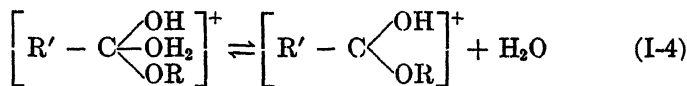
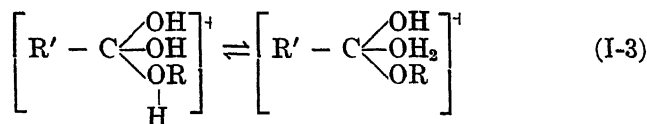
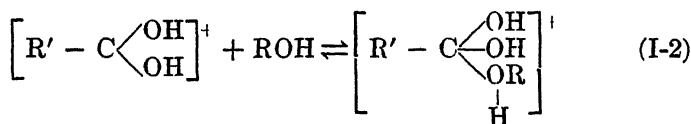
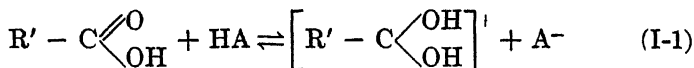
It is possible to eliminate immediately a large number of mechanisms by a consideration of the reactive complex for each of the three reac-

²⁵ Berthelot & Pean de St. Gilles, *Ann. Chim. Phys.* (3) **65**: 335. 1862; **66**: 5. 1862; **68**: 225. 1863. Schultz, *Jour. Am. Chem. Soc.* **61**: 1443. 1939.

²⁶ Kailan & co-workers, *Monatsh.* **51**: 334. 1920; **60**: 386 1932; **61**: 116. 1932; **62**: 284. 1933; **63**: 52. 1933; **63**: 155. 1933

tions under discussion. As Wynne-Jones and Eyring²⁷ have emphasized, kinetics of a reaction alone can only determine the compounds entering into the rate controlling step, *i. e.* the composition of the reactive complex. Granting that the exchange, esterification, and hydrolysis reactions are general acid catalyzed and first order in alcohol or water, their reactive complexes must be, respectively, $R'COOH.H_2O.HA$, $R'COOH.ROH.HA$ and $R'COOR.H_2O.HA$. Of course the last two are identical since the reactive complex must be the same for forward and reverse reactions.

Having determined the composition of the critical complex, it can be stated that all mechanisms which do not form this complex are incorrect. Under this classification falls a group of bimolecular mechanisms (*i. e.* mechanisms consisting of a series of bimolecular steps) several of which have been widely supported. For example, the esterification mechanism of Watson²⁸ although not given as a symmetrical one, may be written



The reverse of this mechanism will be seen to be a similar mechanism for ester hydrolysis.

None of these steps produces the critical complex required by the kinetic data for step (I-1) lacks a molecule of alcohol, steps (I-2), (I-3) and (I-4) lack an A^{-} ion, and step (I-5) lacks a water molecule.

²⁷ Wynne-Jones & Eyring, *Jour. Chem. Phys.* 3: 492. 1935.

²⁸ Evans, Morgan & Watson, *Jour. Chem. Soc.* 1168. 1935. Watson, "Modern Theories of Organic Chemistry", Oxford University Press, 189, 211 1937.

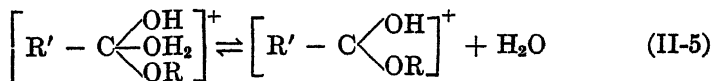
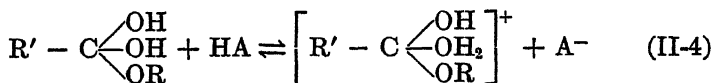
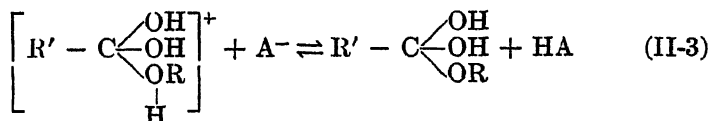
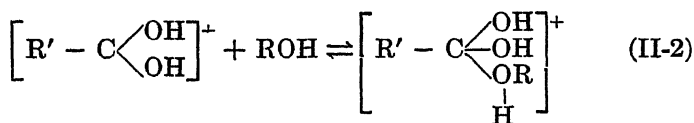
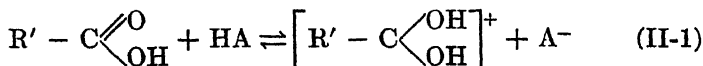
Or, from another point of view, one may state that step I-1 cannot be rate controlling, since the reaction would then be independent of the alcohol concentration, and that step I-2 cannot be rate controlling, for then the reaction would be specific hydrogen ion catalyzed. This last may be seen from the rate expressions

$$V = k_2 [\text{ROH}] \left[\text{R}' - \text{C} \begin{array}{c} \text{OH} \\ \diagup \quad \diagdown \\ \text{OH} \end{array} \right]^+ = k_2 K_1 \frac{[\text{ROH}] [\text{RCOOH}] [\text{HA}]}{[\text{A}^-]} \\ = \frac{k_2 K_1}{K_a} [\text{ROH}] [\text{RCOOH}] [\text{H}^+]$$

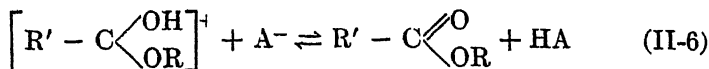
where k_2 is the rate constant for step (I-2), K_1 is the equilibrium constant for step (I-1), and K_a is the dissociation constant of the acid HA. Similar reasoning would apply to the other steps, but this is sufficient to demonstrate that the much simpler consideration of the critical complex involved would eliminate these steps as possibilities.

The mechanism proposed by Ingold^{24, 29} for ester hydrolysis involving the intermediate ion RCO^+ is similarly eliminated since none of its steps contains the correct reactive complex. In fact, on this basis, eight other such bimolecular mechanisms have been eliminated, any of which are as reasonable as the two mentioned above.

There are three possible mechanisms, however, which do lead to the reactive complex required by the kinetics. One of these is a bimolecular mechanism:



²⁹ Ingold & Ingold, Jour. Chem. Soc. 756. 1932.



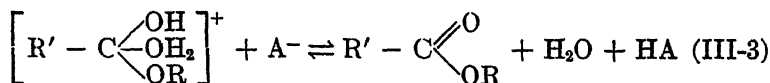
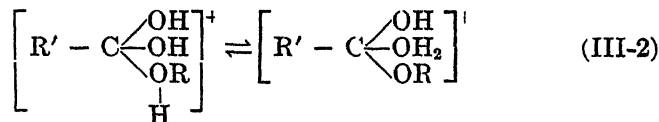
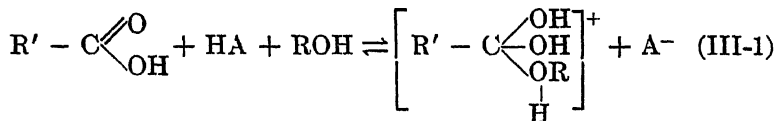
Reaction (II-1) cannot be rate controlling since it does not contain the alcohol. If (II-2) or (II-5) were rate controlling, the reaction would be specific hydrogen ion catalyzed. (II-6) cannot be rate controlling since it does not contain the water. Only (II-3) or (II-4) contain the required reactive complex and hence can be rate controlling. Modifications of this reaction mechanism may be made. Thus instead of (II-1) a reaction donating the proton to the OH group giving $\left[R' - C \begin{array}{c} \text{O} \\ \diagup \\ \text{OH}_2 \end{array} \right]^+$ is possible. This might be followed by

the addition of the ROH to the double bond giving $\left[R' - C \begin{array}{c} \text{OH} \\ \diagup \\ \text{OH}_2 \\ \diagdown \\ \text{OR} \end{array} \right]^+$.

The remainder of the mechanism could then be written in a symmetrical way.

This mechanism II or its modification discussed above, is in line with current mechanisms of similar reactions. From this point of view, the formation of an acid or ester ion by the addition of a proton seems probable. Only after the formation of such an ion or when HA is in the neighborhood of the acid or ester could water or alcohol take part in the reaction since otherwise the reaction would not be catalyzed. The addition and subtraction of protons represented by steps (II-3) and (II-4) are commonly assumed mechanisms.

Lowry³⁰ has proposed a termolecular mechanism for esterification of the following nature:

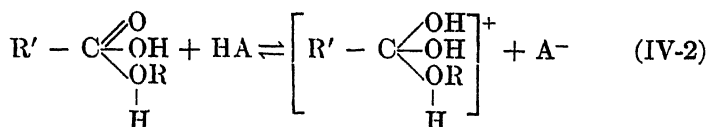
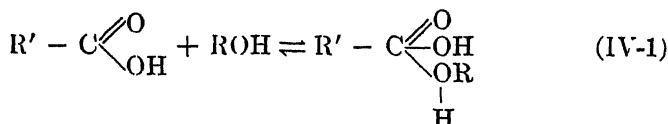


where either step (III-1) or (III-3) may be rate controlling. This

³⁰ Lowry, *Jour. Chem. Soc.* 1381. 1925; *Trans. Faraday Soc.* 24: 545. 1928. **Waters & Lowry**, "Physical Aspects of Organic Chemistry", Van Nostrand, 270-272. 1936

mechanism is entirely possible, since it would yield both general acid catalysis and a first order dependence on the alcohol concentration or on the water concentration in the case of the hydrolysis reaction.

An alternative mechanism would be obtained by separating step (III-1) into the equivalent reactions



in which step (IV-2) would be rate controlling. This differs from mechanism (III) merely in that it supposes a time interval between addition of alcohol and catalyst molecules, rather than a simultaneous addition of the two to the carboxylic acid. In the case of ester hydrolysis this mechanism is lent some support by cryoscopic studies which demonstrate the existence of ester-water compounds in aqueous solution.³¹

The complex formed in step (IV-1) cannot be of the type $R' - C \begin{array}{l} \nearrow OH \\ \searrow \begin{array}{c} OH \\ | \\ OR \end{array} \end{array}$ since this reaction and its reverse would result in uncatalyzed esterification (if a water molecule were removed) as well as rapid uncatalyzed exchange of carboxylic acids. Step (III-3) could be divided into two steps in an entirely similar manner.

The reactive complex method also proves to be more than a method of simplification; it may, in addition, bring out mechanisms which might otherwise have been missed. For example, in the exchange of oxygen between acetone and water, not only is the bimolecular mechanism of Cohn and Urey a possibility, but several other mechanisms, including a termolecular one, will yield the same reactive complex. At the present time, there seems to be no method of distinguishing among these mechanisms for acetone exchange, and also of distinguishing among the mechanisms proposed for the three reactions discussed in this paper. However, the elimination process which we have presented above does lead to a criticism of current theory of the relative rates of acid catalyzed reactions in light and heavy water.

³¹ Kendall & Harrison, *Trans Faraday Soc* 24: 588. 1928

The criterion of pre-equilibrium proposed by Bonhoeffer and Reitz³² is based on a two step mechanism similar to the first two steps of mechanism I. The criterion may be stated as follows: If the rate of an acid catalyzed reaction is increased on going from H_2O to D_2O then a pre-equilibrium exists and the reaction is specific hydrogen ion catalyzed. If, however, the rate is decreased on going from H_2O to D_2O , the first step is not an equilibrium but is rate controlling, and the reaction is general acid catalyzed.

It has been demonstrated that the rate of ester hydrolysis is greater in D_2O than in H_2O for both methyl acetate³³ and ethyl acetate.³⁴ Thus, by the above criterion, we would expect the reaction to be specific hydrogen ion catalyzed. Actually, the data of Dawson quoted above shows that ester hydrolysis is general acid catalyzed. A similar contradiction exists for the esterification reaction. Since the equilibrium constant for the equilibrium ester + water \rightleftharpoons alcohol + acid does not change on going from H_2O to D_2O ,^{35, 36} and since the rate of hydrolysis is known to be greater in D_2O than in H_2O , the rate of esterification must also increase on going from H_2O to D_2O . Here again the data quoted above shows general acid catalysis, whereas the Bonhoeffer criterion would predict a pre-equilibrium.

The source of these difficulties lies in the assumption of a simple two step mechanism, a mechanism which, as we have shown in the discussion given previously, is very improbable for ester hydrolysis and esterification since neither of these steps contains the correct reactive complex.

CONCLUSION

It can be seen that a study of the kinetics and mechanisms of two oxygen exchange reactions has proven very fruitful not only of kinetic data of a new type, but also of information valuable in the consideration of the mechanisms of related organic reactions. It should be emphasized that oxygen exchange reactions proceed at convenient rates with no change in the medium and are of a simple first order dependence. Future kinetic measurements on the reactions discussed should include temperature coefficients and rates in H_2O - D_2O mixtures.

³² Bonhoeffer & Reitz, *Zeit. physikal. Chem. (A)* **179**: 135. 1937. Bonhoeffer, *Trans Faraday Soc.* **34**: 252. 1938. Reitz, *Zeit. Electrochem.* **44**: 72. 1938.

³³ Horne & Butler, *Jour. Chem. Soc.* 1361. 1936.

³⁴ Schwarz, *Zeit. Electrochem.* **40**: 474. 1934.

³⁵ Nachod, *Zeit. Electrochem.* **44**: 72. 1938.

³⁶ Kallan & Ebeneder *Zeit. physikal. Chem. (A)* **180**: 157. 1937.

THE CALCULATION OF EQUILIBRIUM VALUES AND RATE CONSTANTS FOR REACTIONS IN D₂O-H₂O MIXTURES

BY FRANK BRESCIA AND VICTOR K. LAMER

From the College of the City of New York, and Columbia University, New York

The values of hydrogen and deuterium ion concentrations in D₂O-H₂O mixtures, obtainable from kinetic data for a single reaction, may be successfully used to calculate equilibria data. In turn, the equilibrium data may be used to predict the kinetics of other reactions.

An equation, from which the concentration of H⁺ and D⁺ can be calculated, is derived from the results of an investigation, over the entire range of D₂O-H₂O mixtures, of the kinetics of the specific hydrogen ion catalyzed hydrolysis of ethyl orthoformate in acetic acid-sodium acetate buffers of constant ratio, on the basis of the following considerations.

The dissociation constants of acetic acid and of deuterio-acetic acid are assumed to be independent of the deuterium content of the solvent water. This assumption is justified since the dielectric constants and the activity coefficients at constant ionic strength are practically independent of the D content of the solvent. That is

$$K_H = \frac{C_{H^+} \cdot C_{A\bar{c}}}{C_{HAc}} \quad (1)$$

and is constant in any mixture of D₂O-H₂O at constant ionic strength. Similarly,

$$K_D = \frac{C_{D^+} \cdot C_{A\bar{c}}}{C_{DAc}} \quad (2)$$

in any mixture of D₂O-H₂O.

Twice the concentration of acetic anhydride equals the sum of the concentrations of acetic and deuterioacetic acids formed or

$$C_{HAc} + C_{DAc} = 2C_{Ac_2O} \quad (3)$$

On the assumption, previously made by Hamill and LaMer¹ to explain the kinetics of the sucrose inversion in D and H waters, that the velocity in D₂O-H₂O mixtures is proportional to C_{H+} and C_{D+}

$$k = k_H + C_{H^+} + k_D + C_{D^+} \quad (4)$$

¹ Hamill & LaMer, Jour. Chem. Phys. 4: 294. 1936.

where k_{H+} and k_{D+} are the specific catalytic coefficients experimentally determined in $F_D = 0$ and $F_D = 100$ solvents, respectively, and k is the measured rate constant, for buffer ratio 0.001M $\text{Ac}_2\text{O}/0.1\text{M NaAc}$,

(1) F_D	(2) $C_{H+} \times 10^4$	(3) $C_{D+} \times 10^4$	(4) K_4
26.40	3.68	0.624	3.99
55.60	1.93	1.16	3.72
78.49	0.827	1.49	3.46
92.51 ⁴	0.303	1.65	3.86

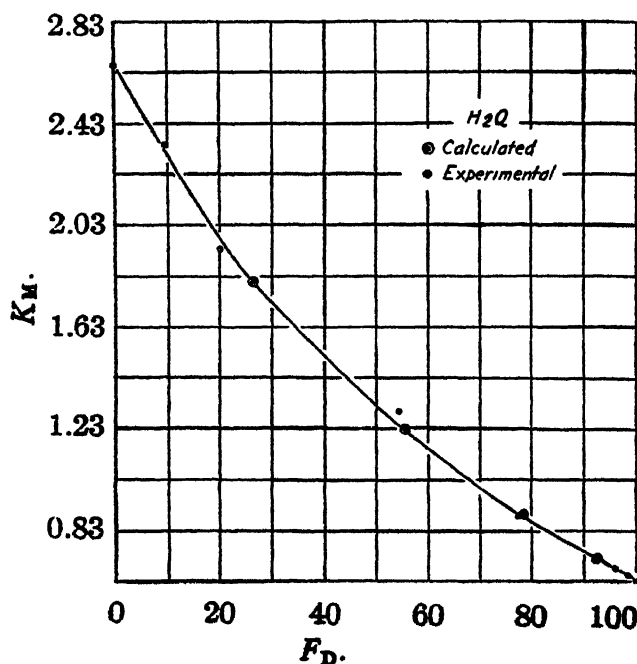


FIGURE 1.

in a water of deuterium content F_D . $F_D = \Delta S(100)/0.1079$. The kinetic measurements were dilatometrically made.

From the above four equations we arrive at the expression:—

$$C_{H+} = \frac{k_{D+} \cdot 2C_{Ac_2O} \cdot K_H}{C_{Ac_2O}(k_{D+} - Kk_{H+})} - \frac{Kk}{k_{D+} - Kk_{H+}} \quad (5)$$

$k_{H+} = 5.93 \times 10^4$; $k_{D+} = 1.39 \times 10^5$; $K_H = 2.87 \times 10^{-5}$, determined

by Harned and co-workers;² $K = K_H/K_D = 3.3$, determined by LaMer and co-workers³ from conductance and e.m.f. measurements.

C_{D^+} is then obtainable from C_{H^+} .

From the values of C_{H^+} and C_{D^+} so obtained from the kinetic data, the equilibrium constant, K_4 , for the process $D^+ + H_2O = H^+ + HDO$ is calculated to be 3.76 (FIGURE 1). Defining the dissociation constant of a weak acid in D_2O - H_2O mixtures as $K_M = (C_{H^+} + C_{D^+}) A^-/HA + DA$, the following equation for the calculation of dissociation constants of weak acids in mixtures of the oxides can then be derived from K_4 :—

$$K_M = K_D \left[1 + 3.76 \frac{C_{H_2O}}{C_{HDO}} \right] / \left[1 + 3.76 \left(\frac{K_D}{K_H} \right) \frac{C_{H_2O}}{C_{HDO}} \right] \quad (6)$$

where K_M is the dissociation constant of the acid in the mixtures of H_2O - D_2O , K_D is the dissociation constant of the heavy acid, and K_H is the dissociation constant of the light acid.

The K_M values, calculated from equation 6, are in very good agreement with the experimental results.⁴ This agreement for hydroquinone⁴ is shown in the figure one and it is equally good for acetic and benzoic acids.⁴ It is apparent that true values of hydrogen and deuterium ion concentrations are obtainable from kinetic data when tested by means of acid dissociation constants. This also constitutes proof of the validity of the basic assumption that for a specific hydrogen ion catalyzed reaction, the rate in D_2O - H_2O mixtures should be proportional to the relative concentrations of D^+ and H^+ .

It is also possible to predict the rate constants for specific hydrogen ion catalyzed reactions in mixtures of the oxides from

- (a) the equilibrium equation $D^+ + H_2O = H^+ + HDO$; $K_4 = 3.76$
- (b) the equation $C_{D^+} + C_{H^+} = \text{concentration of strong acid in any oxide mixture}$
- (c) the previous hypothesis that the rate is proportional to F_{D^+} .

Similar predictions may be made for specific hydroxyl ion catalyzed reactions from the corresponding equations:—

- (a') $OH^- + HDO = OD^- + H_2O$; $K_5 = 0.225$

² Harned & Robinson, Jour. Am. Chem. Soc. 50: 3157. 1928 Harned & Owen, Jour. Am. Chem. Soc. 52: 5079. 1930.

³ Korman & LaMer, Jour. Am. Chem. Soc. 58: 1396. 1936. Chittum & LaMer, Jour. Am. Chem. Soc. 59: 2425. 1937.

⁴ Chittum & LaMer, cf. ref. 3. Rule & LaMer, Jour. Am. Chem. Soc. 60: 1974. 1938.

(b') $C_{OH-} + C_{OD-}$ = concentration of alkali in any oxide mixture

(c') $k = k_{OH-} C_{OH-} + k_{OD-} C_{OD-}$

where k_{OH-} and k_{OD-} are the specific rate constants in $F_D = 0$ and

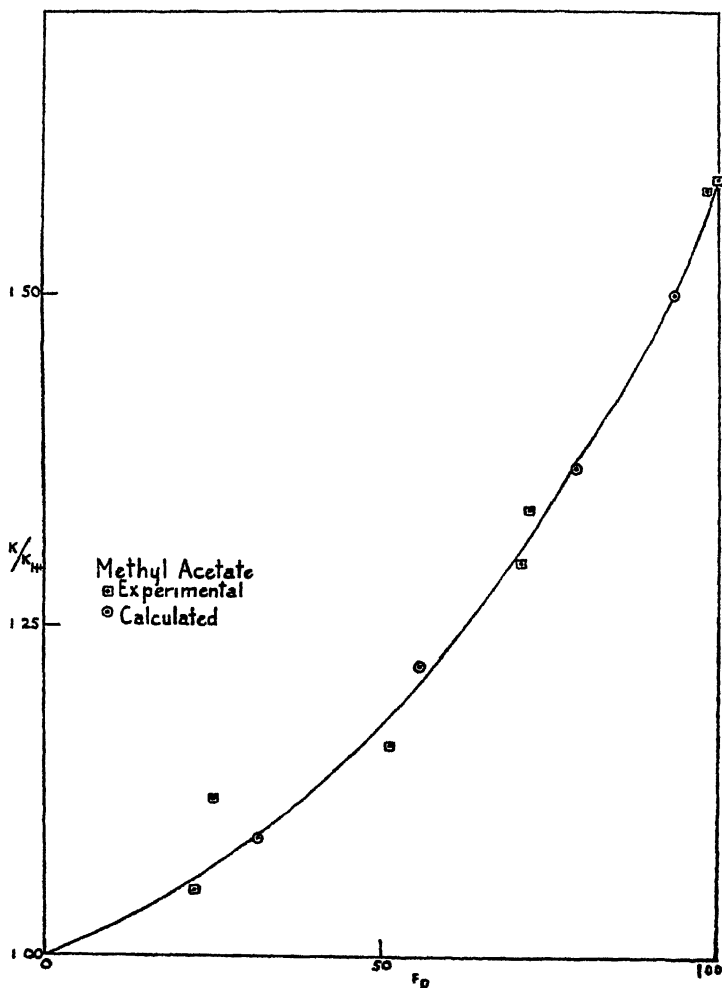
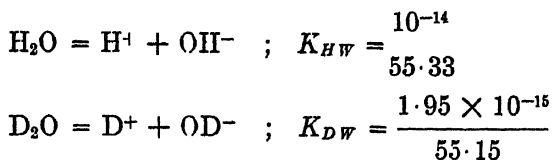


FIGURE 2.

$F_D = 100$ solvent and k is the rate constant at different intermediate fractions of D.

The value of K_s is derived from the equilibrium constant 3.27, cal-

culated by Topley and Eyring,⁵ for the process $\text{H}_2\text{O} + \text{D}_2\text{O} = 2\text{HDO}$, from K_4 (3.76), and from the equilibrium constants of the following processes, determined by Wynne-Jones:⁶—



The predicted rates are in good agreement with the available experimental data for specific hydrogen ion catalyzed reactions in which the rate is faster in D_2O —namely, the hydrolysis of methyl acetate, acetal, and ethyl formate studied over a range of D and H waters by Butler and co-workers.⁷ This agreement is illustrated in FIGURE 2 with the methyl acetate reaction. Very poor agreement is obtained in the sucrose inversions⁸ and glucose mutarotation⁹ reactions. In the mutarotation of glucose reaction, which is exceptional in that the k_{D+} is smaller than the k_{H+} , the theoretical curve shows a positive deviation from linearity whereas the best curve through the experimental data is a straight line. The calculated results are also in very poor agreement with the experimental curve for the alkaline decomposition of diacetone alcohol investigated by Nelson and Butler.⁷ A definite sag in the theoretical curve is observed whereas the available data indicates, again, a straight line relation between k and F_D .

As suggested by Hamill and LaMer in the case of glucose, it is quite possible that the lack of agreement in the sucrose, glucose, and diacetone alcohol reactions may be due to substrate exchange with the solvent. The preliminary results of Hochberg and LaMer seem to indicate the possibility that the rate of the base catalyzed reaction of nitramide decomposition is a linear function of F_D . Nitramide also exchanges with the solvent.

⁵ Topley & Eyring, *Jour. Chem. Phys.* 2: 217. 1934.

⁶ Wynne-Jones, *Trans. Faraday Soc.* 32: 1397. 1936.

⁷ Orr & Butler, *Jour. Chem. Soc. London* 330 1937. Nelson & Butler, *Jour. Chem. Soc.* 937 1938.

⁸ Moelwyn-Hughes & Bonhoeffer, *Naturwissen* 22: 174. 1934. Gross, Suess & Steiner, *Naturwissen* 22: 662 1934. Gross, Steiner & Suess, *Trans. Faraday Soc.* 32: 883. 1936.

⁹ Hamill & LaMer, *Jour. Chem. Phys.* 4: 395. 1936.

THE KINETICS OF SOME AMINE CATALYZED REACTIONS¹

BY F. H. WESTHEIMER

From the George Herbert Jones Laboratory of the University of Chicago

There are many reactions, like the saponification of an ester, which are catalyzed by hydroxide ion or in which hydroxide ion enters directly. There are, on the other hand, a few reactions which are catalyzed by bases in general, that is, by any molecule which can act as a proton acceptor. For example, in the decomposition of nitramide, discovered by Brönsted and Pedersen² in 1924, not only hydroxide ion, but such bases as aniline and acetate ion are catalysts. It is characteristic of such a reaction that, at constant ionic strength and constant buffer ration (and therefore at constant hydroxide ion concentration) the rate of the reaction increases linearly with buffer concentration. The rate of the reaction can accurately be expressed by the equation:

$$k_{\text{obs}} = \Sigma k_B(B)$$

where k_{obs} is the observed first order rate constant, and k_B is the catalytic constant of the base at the concentration (B). Further, there is a relationship between the strengths of the bases used as catalysts and the corresponding rate constants:

$$k_B = GK_B^x$$

Here G is a constant for a series of bases of similar charge type and structure, K_B is the dissociation constant of the base, and $0 < x < 1$.

Recently a third type of reaction in alkaline solution, namely catalysis by primary and secondary amines, has been discovered. While this type of catalysis has been established by kinetic studies only for the decarboxylation of acetoacetic acid, for the decarboxylation of dimethyl acetoacetic acid and for the dealdolization of diacetone alcohol, there is qualitative evidence that other reactions probably belong in the same category. For example, Fischer and Marshall³ found that alanine catalyzes the condensation of acetaldehyde in the

¹ Presented at the Conference on Kinetics in Solution, The New York Academy of Sciences, on February 24, 1939. Revised August 1, 1939.

² Brönsted & Pedersen, *Zeit physikal. Chem.* 103: 185. 1924. Brönsted, *Chem. Rev.* 5: 231. 1928.

³ Fischer & Marschall, *Ber.* 64B: 2825 1931.

presence of phosphate buffers at pH 7, and in the reaction of nitromethane with benzaldehyde, the product Worall⁴ obtained with a primary or secondary amine differed qualitatively from that found when a tertiary amine was used as catalyst. The present paper is devoted to a detailed discussion of the cases in which the kinetics of amine catalysis is known.

There have been several quantitative studies of the decarboxylation of acetoacetic acid. Ljunggren⁵ found that both the acid and the salt lose carbon dioxide, the former much more rapidly than the latter. The rate of the reaction, in the absence of amines, can accurately be represented as the sum of the two independent reactions, the decomposition of the acid and that of the salt. The reaction is markedly accelerated by aniline, while dimethyl aniline is without effect on the rate. Since both amines have about the same strength, general base catalysis is out of the question.

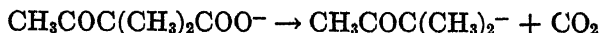
Recently, Pedersen⁶ has made a careful study of the decarboxylation of dimethyl acetoacetic acid, both in the presence, and in the absence of amines. Using the dimethyl derivative of acetoacetic acid has the advantage that enolization of the acid need not be considered in the mechanism.

Both the acid and the ion lose carbon dioxide, and, as in the case of the unsubstituted compound, the rate at which the acid loses carbon dioxide greatly exceeds the rate for the ion. In the absence of amines, the rate is given by the expression:

$$\frac{d(\text{CO}_2)}{dt} = k_{\text{acid}} (\text{Acid}) + k_{\text{ion}} (\text{Ion})$$

That the reaction is not general base catalyzed is shown by the fact that glycolate ion is without effect on the rate.

The decomposition of the ion can be represented by the equation:



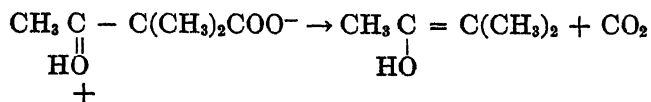
where $\text{CH}_3\text{COC}(\text{CH}_3)_2^-$ is the enolate ion of methyl isopropyl ketone; the enolate ion reacts rapidly with water to yield the corresponding ketone. An adequate mechanism for the decarboxylation of the acid itself is more difficult to formulate. To explain the decarboxylation of the acid (and also to account for the amine catalysis), Pedersen tentatively advanced the ingenious hypothesis that the compound

⁴ Worral, *Jour. Am. Chem. Soc.* **56**: 1556, 1934.

⁵ Ljunggren, *Dissertation, Lund*. 1925, *Ber ges Physiol* **38**: 620, 1927.

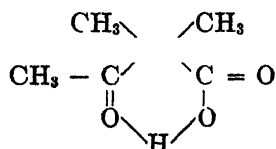
⁶ Pedersen, *Jour. Am. Chem. Soc.* **51**: 2098, 1929, **58**: 240, 1936, **60**: 595, 1938; *Jour. Phys. Chem.* **38**: 559, 1934.

which loses carbon dioxide is not the acid itself, but rather the corresponding zwitterion. That is, Pedersen assumed that the decarboxylation of dimethyl acetoacetic acid leads directly to the enol.



That the enol is actually produced, Pedersen showed by carrying out the reaction in the presence of bromine and iodine. Under the conditions of the reaction, the rate of halogenation of methyl isopropyl ketone is small, but the rate of disappearance of halogen is identical with the rate at which the decarboxylation takes place in the absence of the halogen. In other words, the decarboxylation leads directly to a compound which brominates rapidly compared with the rate of bromination of methyl isopropyl ketone itself. In view of present knowledge of the bromination reaction⁷ it seems most likely that this reactive intermediate is the enol. Further, the stability of ketopinic acid has led Bret⁸, somewhat earlier, to suggest that an enol is directly produced in decarboxylation.

The mechanism advanced by Pedersen is not, of course, the only one which would account for the formation of the enol as the primary reaction product. A mechanism equally in accord with the kinetic evidence would replace the zwitterion by a chelate ring structure,



or by the cyclic ester of the hydrated form of the ketone. Experimental work which may help decide among these various possibilities is now in progress at the University of Chicago.

In the presence of amines the rate expression is quite complicated. In order to account accurately for the rate of the aniline catalyzed decarboxylation, Pedersen was forced to employ the following equation:

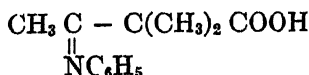
$$\frac{d(\text{CO})_2}{dt} = k_{\text{acid}} (\text{Acid}) + k_{\text{ion}} (\text{Ion}) + k_B (\text{Aniline}) (\text{Acid}) + k_{B'} (\text{Aniline})^2 (\text{Acid})$$

Since more than one interpretation is possible, the exact mechanism is

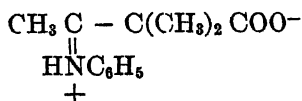
⁷ Wilson, *Trans. Faraday Soc.* **34**: 175. 1938.

⁸ Bret⁸, *C* **98**: 11, 2298. 1927.

open to doubt. In solutions of low aniline concentration, only the first three terms need be considered. Pedersen pointed out the concentration both of the Schiff base



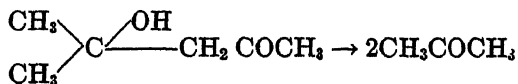
and of the zwitterion



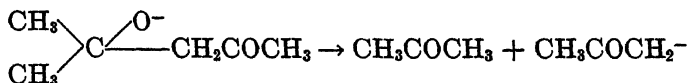
will be proportional to the product of the concentrations of aniline and acid. The strength of the Schiff base will greatly exceed that of the keto acid, due to the greater basicity of a nitrogen as compared with an oxygen atom. Despite the fact that the concentration of aniline is low, and the formation of the Schiff base incomplete, the concentration of the zwitterions arising from the Schiff base may well exceed that arising from dimethyl acetoacetic acid itself. On the basis of the reasonable assumption that the rates of the decarboxylation of the two dipolar ions are comparable, the catalytic effect of primary amines on the decarboxylation of dimethyl acetoacetic acid can be explained.

This explanation is by no means unique; in addition to alternative mechanisms similar to those advanced in connection with the uncatalyzed decarboxylation of dimethyl acetoacetic acid, it is quite possible that the rate controlling step is the formation of the ketimine.

Before considering the effect of amines on the dealdolization of diacetone alcohol, it is necessary to establish the mechanism in the presence of sodium hydroxide. The reaction



is strictly first order with respect to the concentration of diacetone alcohol, and the rate varies linearly with the hydroxide ion concentration. The evidence to show that the reaction is not subject to general base catalysis will be presented shortly. Accepting this conclusion, the most probable mechanism for the reaction involves the unimolecular decomposition of the diacetone alcoholate ion. Since the alcohol is undoubtedly a very weak acid, the concentration of this ion will be strictly proportional to the hydroxide ion concentration. In the equation for the decomposition of the diacetone alcoholate ion which follows, $\text{CH}_3\text{COCH}_2^-$ is the enolate ion of acetone.



The enolate ion subsequently reacts with water to give acetone and hydroxide ion.

The fact that the dealdolization is catalyzed by amines was first demonstrated by Miller and Kilpatrick.⁹ Using solutions of ammonia and ammonium chloride, for example, they found that, at constant ionic strength and constant buffer ratio, the rate of the reaction increases linearly with increasing buffer concentration. The rate can be expressed by the equation,

$$k_{\text{obs}} = k_{\text{OH}^-}(\text{OH}^-) + k_{\text{B}}(\text{B})$$

where k_{obs} is the first order rate constant for the dealdolization in the particular solution chosen, k_{OH^-} the specific rate constant for catalysis by hydroxide ions present at a concentration (OH^-) and k_{B} is the rate constant for the catalysis by the amine, present at a concentration (B) . French,¹⁰ however, had previously shown that the reaction is not catalyzed by phenolate ion, a base in the Brönsted sense of the term. Further, the rate constants for the various amines investigated by Miller and Kilpatrick bear no simple relation to the base strengths of the amines. Stimulated by this interesting work by Miller and Kilpatrick, a further investigation of the amine catalysis of the dealdolization of diacetone alcohol was carried out by Herzl Cohen and the author.¹¹ The reaction, while catalyzed markedly by molecular methyl amine and to some extent by dimethyl amine, is quite unaffected by trimethyl or triethyl amine (although, of course, the reaction is catalyzed by the hydroxide ions present in the solution). Figure 1 shows the results of this investigation. Here each line refers to a particular buffer ratio of an amine and its hydrochloride. The increase in rate with increasing buffer concentration with methyl and dimethyl amine is quite clear. The intercept on the rate axis for zero buffer concentration refers to the reaction produced by the hydroxide ions. Increasing concentrations of buffers of tertiary amines produce no change in rate, and this fact, in conjunction with French's observation that phenolate ion does not catalyze the reaction, clearly demonstrates that the dealdolization of diacetone alcohol exhibits specific amine, and not general base catalysis. Since tertiary amines will not

⁹ Miller & Kilpatrick, *Jour. Am. Chem. Soc.* **53**: 3217. 1931.

¹⁰ French, *Jour. Am. Chem. Soc.* **51**: 3215. 1929.

¹¹ Westheimer & Cohen, *Jour. Am. Chem. Soc.* **60**: 90. 1938.

SUMMARY

The kinetics of the decarboxylation of dimethyl acetoacetic acid and of the dealdolization of diacetone alcohol, both in the presence and in the absence of amines have been discussed. Several mechanisms have been suggested for the amine catalyzed dealdolization. While the investigation of neither reaction is complete, present work shows that, in addition to hydroxide ion catalysis and general base catalysis, a specific amine catalysis must be considered in discussing the mechanisms of organic reactions.

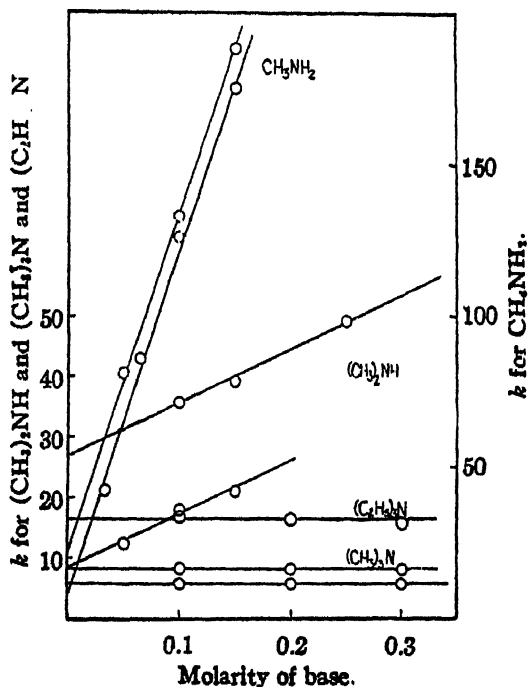


FIGURE 1.

